UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

DATE: **April 15, 2019**

SUBJECT: **Pethoxamid:** Report of the Cancer Assessment Review Committee

PC Code: 090208
Decision No.: 528729
Petition No.: N/A
Risk Assessment Type: NA
TXR No.: 0057851
MRID No.: N/A

DP Barcode: D449604
Registration No.: 279-GAEO
Regulatory Action: Section 3 registration
Case No.: N/A
CAS No.: 106700-29-2
40 CFR: N/A

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On October 24, 2018, the Cancer Assessment Review Committee (CARC) of the Health Effects Division, evaluated the carcinogenic potential of pethoxamid in accordance with the *EPA's Final Guidelines for Carcinogen Risk Assessment* (March, 2005). The final Cancer Assessment Document is attached.

CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF

PETHOXAMID

FINAL REPORT

April 15, 2019

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
Office of Pesticide Program

Contents

EXECUTIVE SUMMARY

On October 24, 2018, the Cancer Assessment Review Committee (CARC) of the Health Effects Division (HED) of the Office of Pesticide Programs (OPP) met to evaluate the carcinogenic potential of pethoxamid. In accordance with the 40 CFR Part 158 Toxicology Data requirements, long-term studies [i.e., chronic/carcinogenicity study in rats (MRID 49575317) and carcinogenicity study in mice (MRID 49575318)] were submitted for review to support the new active ingredient (a.i.) registration. In addition, the Registrant submitted mechanistic studies to support a proposed mode of action for the observed liver and thyroid tumors in rats and mice.

The CARC considered the following in its weight-of-evidence determination of the carcinogenic potential of pethoxamid:

Rats

In a carcinogenicity study (MRID 49575317), groups of 80 Crl:CD BR rats/sex/dose were administered pethoxamid (TKC-94; 95.0% a.i.; Lot No. TB-960306) in the diet at concentrations of 0, 25, 400, or 1600 ppm (equivalent to 0/0, 1.0/1.4, 17.0/23.3, and 70/99 mg/kg/day in males/females) for up to 105 weeks. Interim sacrifices were conducted on ten rats/sex/dose level at Weeks 26, 52, and 78.

- With regards to survival, there were no disparities in females and a negative pair-wise comparison of the low dose group with controls in males.
- No significant increase in the incidence of tumors was observed in female rats. Male rats had statistically significant trends for thyroid follicular cell adenomas at $p < 0.01$. Also, there was a statistical difference in pair-wise comparison for adenomas at 1600 ppm ($p<0.05$). Thyroid adenomas were considered treatment-related in males at 1600 ppm, the highest dose tested (HDT).
- Relevant non-neoplastic findings included male thyroid hyperplasia (not significant) at 1600 ppm.
- The CARC concluded that dosing was adequate and not excessive for the carcinogenicity study in the rat based on the treatment-related tumors as well as thyroid enlargement, thyroid hyperplasia, cystic degeneration and clear cell hepatocytes observed at the highest dose tested in males.

The registrant submitted mechanistic studies and a mode of action (MOA) proposal for pethoxamid-induced rat thyroid tumors through activation of the constitutive androstane receptor (CAR). **The CARC concluded that the submitted data do not adequately support the proposed MOA** based on the following considerations:

- Key event #1 (CAR activation): Increased thyroxine glucuronidation and UGT were observed at 1600 ppm. UGT1A6 mRNA levels were significantly increased starting at 400 ppm. These effects were reversible after a 42-day recovery period.

- Key event #2 (induction of replicative DNA synthesis): BrdU labeling was marginally increased at 1600ppm but this increase was not statistically significant. Thus, the induction of replicative DNA synthesis is considered equivocal. Also, no significant changes in thyroid hormones (T3, T4 or TSH) were reported in the mechanistic studies.
- Key event #3 (formation of adenomas): male thyroid adenomas were observed at 1600 ppm.
- Associative events: In rats, there was a significant increase in thyroid follicular cell hypertrophy at 1600 ppm in males following treatment with pethoxamid for 7 days (MRID 49813533) and 90 days (MRID 49575314). However, no thyroid hypertrophy was observed in the chronic study in rats.

Mice

In a carcinogenicity study (MRID 49575318), groups of 60 CD-1 (Crl:CD-1 BR) mice/sex/dose group were administered pethoxamid (TKC-94; 94.8-95.0% a.i.; Lots TB-960306 and TB 960306C) in the diet at dose levels of 0, 30, 400, or 5000 ppm (equivalent to 0/0, 4.0/5.0, 56.8/68.0, and 982/1068 mg/kg/day in males/females) for up to 95 weeks in males and 92 weeks in females. Interim sacrifice groups of 10 mice/sex/dose group were euthanized at 52 weeks.

- With regards to survival, there were no disparities in females and a negative trend ($p<0.05$) in control males.
- No significant increase in the incidence of tumors was observed in female rats. Male mice had statistically significant trends, and statistically significant pair-wise comparisons of the 5000 ppm dose group with the controls, for liver adenomas and combined adenomas and carcinomas, all at $p < 0.01$.
- Relevant non-neoplastic findings included statistically significant increases in hepatocellular hypertrophy in males and females at 5000 ppm (HDT).
- The CARC concluded that dosing was adequate and not excessive for the carcinogenicity study in the mouse based on the treatment-related tumors in males as well as microscopic findings (swelling/rarefaction of the villous epithelium) in the duodenum and jejunum and decreased body weights in males and females observed at the highest dose tested (limit dose).

The registrant submitted mechanistic studies and a MOA proposal for pethoxamid-induced mouse liver tumors through activation of the CAR. **The CARC concluded that the submitted data adequately support the proposed MOA** based on the following considerations:

- Key event #1 (CAR activation): Increased PROD was observed at 1200 ($p<0.05$) and 5000 ppm ($p<0.01$), increased CYP mRNA at 400 (CYP2B10; $p<0.05$) and 5000 ppm (CYP1A2, CYP2B10, CYP3A11, CYP4A10; $p<0.05$) and increased CYP activity at 5000 ppm (CYP1A, CYP2B, CYP3A2, CYP4A1; $p<0.05$).
- Key event #2 (induction of replicative DNA synthesis): There was a statistically significant increase in BrdU ($p<0.001$) and PCNA labeling ($p<0.05$) at 1600ppm.

- Key event #3 (formation of liver tumors): male liver adenomas and combined adenomas and carcinomas were observed at 5000 ppm.
- Associative events: liver hypertrophy and increased liver weights were reported in multiple studies.

In accordance with the EPA's Final Guidelines for Carcinogen Risk Assessment (March, 2005), the CARC classified pethoxamid as **"Suggestive Evidence of Carcinogenic Potential"** based on male rat thyroid follicular cell adenomas. There is insufficient evidence to support the proposed thyroid tumor MOA in male rats. The MOA proposal for pethoxamid-induced mouse liver tumors through activation of the CAR was found to be acceptable. There is no concern for mutagenicity.

I. BACKGROUND INFORMATION

Pethoxamid is a new a.i. being proposed for use as an emulsifiable concentrate preplant, preplant incorporated, preemergence, and/or early post-emergence weed control herbicide. The proposed crop use sites include soybean, cotton, field corn, sweet corn, and popcorn. Pethoxamid is also proposed to be used in/on turf and ornamental sites in residential, commercial, and institutional lawns and landscapes, golf courses, sod farms, utility right-of-ways, roadsides, railways, industrial areas, and container and field grown ornamentals. It can be applied via aerial, ground, and chemigation application with application rates that range from 1.5 to 3 lbs ai/acre. Proposed pethoxamid products may also be used for impregnation of bulk fertilizer.

Pethoxamid is a chloroacetamide (see Figure 1) herbicide. Its proposed pesticidal mode of action (MOA) is inhibition of Group 15 Very Long Chain Fatty Acid Elongase (VLCFAE) in plants. The mammalian MOA for toxicity is not known at this time. The registrant has proposed a MOA for liver tumors in the mouse and thyroid tumors in the rat (see Section 1V).

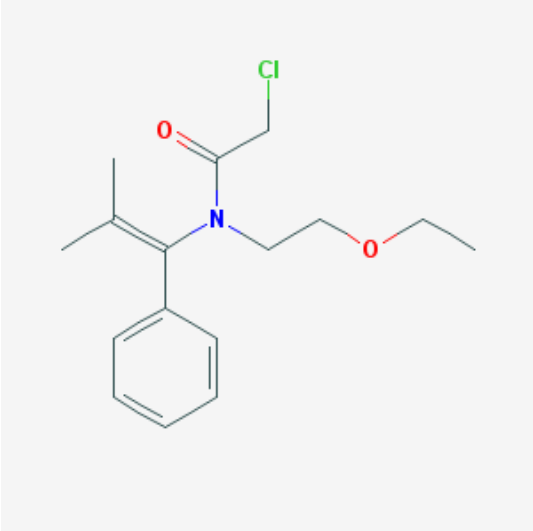

Figure 1. Structure of Pethoxamid

II. EVALUATION OF CARCINOGENICITY STUDIES

A. Combined Chronic Toxicity/Carcinogenicity Study in Rats

Citation: Waterson, L.A. (2001) TKC-94: Potential tumorigenic and toxic effects in prolonged dietary administration to rats. Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, England. Laboratory Experiment No.: TON 6/974064, January 15, 2001. MRID 49575317. Unpublished.

Experimental Design:

In a carcinogenicity study (MRID 49575317), groups of 80 Crl:CD BR rats/sex/dose were administered pethoxamid (TKC-94; 95.0% a.i.; Lot No. TB-960306) in the diet at concentrations of 0, 25, 400, or 1600 ppm (equivalent to 0/0, 1.0/1.4, 17.0/23.3, and 70/99 mg/kg/day in

males/females) for up to 105 weeks. Interim sacrifices were conducted on ten rats/sex/dose level at Weeks 26, 52, and 78.

1. **Survival analysis:**

Male rats had a statistically significant difference (negative pair-wise) between the control and low dose groups for mortality, at $p < 0.05$ (Table 1). There were no statistical differences in mortality for female rats.

Table 1. Pethoxamid – Crl:CD BR Rat Study (MRID No. 49575317)
Male Mortality Rates[+] and Cox or Generalized K/W Test Results

	Weeks							
Dose (ppm)	1-26	27[i]	27-52	53[i]	53-78	79[i]	79-105[f]	Total
0	1/80	10/79	4/69	10/65	10/55	10/45	16/35	31/50 (62)
25	0/80	10/80	0/70	10/70	8/60	9/52	16/43	24/51[*n] (47)
400	0/80	10/80	4/70	10/66	11/56	4/45	26/41	41/56 (73)
1600	0/80	10/80	2/70	10/68	10/58	8/48	13/40	25/52 (48)

+Number of animals that died during the interval/Number of animals alive at the beginning of the interval.
[i]Interim sacrifices at weeks 27, 53 and 79.
[f]Final sacrifice at week 105.
[n]Negative pair-wise comparison of the control with the 25-ppm dose group.

Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If [*], then $p < 0.05$. If [**], then $p < 0.01$.

2. **Tumor Analyses:**

No significant increase in the incidence of tumors was observed in female rats. Male rats had statistically significant trends for thyroid follicular cell adenomas at $p < 0.01$. There was also a statistically significant pair-wise comparison of the 1600 ppm dose group with the controls for thyroid follicular cell adenomas at $p < 0.05$, which was outside of historical control data. The statistical analyses of the tumors in the male rat study were based upon Peto's Prevalence Test (Table 2) (L. Brunsman, 9/26/2018, TXR 0057789).

Table 2. Pethoxamid – Crl:CD BR Rat Study (MRID No. 49575317)
Male Thyroid Follicular Cell Tumor Rates[+] and Peto's Prevalence Test Results

	Dose (ppm)				
	0	25	400	1600	Historical control^
Adenomas (%)	2/54 (4)	2/60 (3)	2/54 (4)	9[a]/55 (16)	0-12%
P =	0.00117**	0.60413	0.62943	0.03172*	
Carcinomas (%)	2[b]/19 (11)	0/27 (0)	0/15 (0)	0/27 (0)	0-5.1%
P =	0.87486[n]	0.95589[n]	0.89903[n]	0.95589[n]	

+Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.
^Studies starting between 1994 and 2005
[a]First adenoma observed at week 64 in the 1600 ppm dose group.
[b]First carcinoma observed at week 105 in the control group during the final sacrifice.
[n]Negative trend or negative pair-wise comparison of the dosed groups with the control.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

3. Non-neoplastic Lesions and Other Findings:

Body weights were decreased (not statistically evaluated) in the main animals from 7-11% in males and 6-19% in females at 1600 ppm from Week 1 to Week 104. For the main group animals, this was most pronounced in the period of Weeks 0 to 88, with differences relative to control lessening from Weeks 88 to 104 (Table 3a).

Mean relative (to body weight) liver weights were increased by 14-19% at the interim sacrifices, and at Week 104 for males (Table 3b). In females, mean relative (to body weight) liver weights were increased by 16-19% at Weeks 26, 78, and 104. Generally non-dose-dependent mean relative (to body weight) thyroid weight effects were apparent in treated males, but not females, at Weeks 26, 52, and 78. An apparent decrease in relative (to body weight) thyroid weights at Week 104 was abolished after removal of a single control group value that was an outlier.

At Week 104, treatment-related gross pathological findings (Table 4) in the 1600 ppm animals included: thyroid enlargement (12/50 treated vs. 4/50 control) and enlarged liver (6/50 treated vs. 2/50 control) in males only. A treatment-related increase in the incidence of centrilobular hepatocyte hypertrophy was seen in the main group male and females (11/50 and 8/50, respectively treated vs. 0/50 controls). This finding was considered associated with the increased mean body weight-adjusted liver weights noted for male and females, as well as the increased incidence of liver enlargement reported macroscopically for males. Treatment-related incidences of centrilobular hepatocyte hypertrophy also were seen in satellite males and females at Week 26, and in satellite males at Weeks 52 and 78. Additionally, increased incidences of hepatocytes

with concentric intracytoplasmic inclusions (mainly in periportal zones), focal cystic degeneration, and focal clear cell hepatocytes were observed in males. Increased incidences of hepatocytes with concentric intracytoplasmic inclusions (mainly in periportal zones) also were noted at Weeks 26 and 52 in the satellite males.

TABLE 3a. Mean (±SD) body weights and mean (±SD) body weight gains (g) at selected intervals in rats administered pethoxamid in the diet for up to 104 weeks in the main groups. [a]

Weeks	Concentration (ppm)			
	0	**25**	**400**	**1600**
Males				
0	226±13.2	223±14.3	224±14.9	224±14.5
2	332±25.5	331±28.5	327±26.7	309±27.4 (↓7)
4	402±35.1	402±41.9	394±34.8	374±35.5 (↓7)
26	663±78.4	665±82.4	639±65.3	598±76.6 (↓10)
52	773±94.8	776±100.5	759±93.2	712±106.8 (↓8)
78	848±104.5	845±116.2	811±112.0	755±112.0 (↓11)
88	856±108.8	857±118.4	836±95.7	770±101.2 (↓10)
104	785±87.6	800±126.4	760±97.7	730±108.6 (↓7)
BWG (0-4)	176±27.5	178±32.4	170±25.0	149±25.9** (↓15)
BWG (4-88)	460±94.3	460±98.0	442±84.8	398±86.3** (↓13)
BWG (0-88)	631±106.6	635±114.3	612±93.4	547±96.5** (↓13)
BWG (88-104)	−53±71.3	−44±66.3	−44±51.1	−40±54.8
BWG (0-104)	560±87.5	579±120.8	540±99.2	506±101.9 (↓10)
Females				
0	167±11.2	164±11.1	163±11.3	163±11.2
2	210±16.0	209±16.5	206±17.6	198±15.4 (↓6)
4	242±20.3	241±21.1	237±21.0	226±18.4 (↓7)
26	342±49.4	342±44.5	320±37.9 (↓6)	308±31.4 (↓10)
52	439±96.6	435±70.5	401±64.8 (↓9)	367±31.4 (↓16) [b]
78	527±123.5	520±75.2	483±93.9 (↓8)	427±67.5 (↓19)
88	543±134.6	540±82.2	501±119.9 (↓8)	454±75.3 (↓16)
104	473±103.9	534±73.4	516±64.0	447±84.1 (↓5)
BWG (0-4)	75±12.7	77±13.4	74±12.9	64±10.3** (↓15)
BWG (4-88)	303±121.7	299±75.4	261±108.5 (↓14)	226±67.3** (↓25)
BWG (0-88)	376±131.4	377±79.6	336±116.2 (↓11)	290±70.0** (↓23)
BWG (88-104)	−28±91.8	−2±56.5	−16±67.0	−2±59.1
BWG (0-104)	308±99.1	375±71.1	349±65.9	283±80.5 (↓8)

a Data were obtained from Appendix 1 on pages 260-371 of MRID 49575317; SD for body weights calculated by the reviewers from individual data. Percent difference from control is included in parentheses (calculated by reviewers for body weights only).

b Mean reported as 368 in Table2 on pages 54 of MRID 49575317.

** Significantly different ($p \leq 0.01$) from control.

TABLE 3b. Selected mean (±SD) absolute and relative (to body weight) organ weights in rats administered pethoxamid in the diet for up to 104 weeks. [a]

Parameter	Concentration (ppm)			
	0	25	400	1600
Males				
Week 26 BW(g)	697±84.8	673±87.7 (↓3)	668±58.6 (↓4)	580±63.4** (↓17)
Week 26 Absolute Liver (g)	25.7±3.11	24.0±2.95 (↓6)	24.7±2.36 (↓4)	24.6±3.12 (↓4)
Week 26 Relative (to BW) Liver	3.71±0.32	3.58±0.31	3.70±0.26	4.25±0.28** (↑15)
Week 26 Absolute Kidney (g)	4.03±0.423	3.97±0.495	4.07±0.314	3.58±0.470
Week 26 Relative (to BW) Kidney	0.58±0.07	0.61±0.11	0.61±0.03	0.62±0.06
Week 26 Absolute Thyroid (mg)	27.2±5.51	28.5±5.07	25.9±7.47	25.8±5.93 (↓5)
Week 26 Relative (to BW) Thyroid	3.91±0.78	4.16±1.30	3.86±0.91	4.42±0.68 (↑13)
Week 52 BW (g)	790±116.1	762±85.2	698±106.3	709±106.2 (↓10)
Week 52 Absolute Liver (g)	26.7±4.06	25.7±4.20	23.2±4.03	27.2±3.79 (↑2)
Week 52 Relative (to BW) Liver	3.38±0.24	3.36±0.41	3.33±0.38	3.86±0.41* (↑14)
Week 52 Absolute Kidney (g)	4.11±0.422	4.60±0.539	4.32±0.482	4.03±0.501
Week 52 Relative (to BW) Kidney	0.53±0.06	0.61±0.06* (↑15)	0.63±0.07** (↑19)	0.57±0.05 (↑8)
Week 52 Absolute Thyroid (mg)	29.6±8.51	38.3±10.74 (↑29)	35.3±9.67 (↑19)	38.7±9.61 (↑31)
Week 52 Relative (to BW) Thyroid	3.72±0.85	5.01±1.16 (↑35)	5.06±1.18* (↑36)	5.47±1.11** (↑47)
Week 78 BW (g)	813±165.0	769±114.7	747±72.6	764±100.3 (↓6)
Week 78 Absolute Liver (g)	25.9±6.78	23.3±3.15	25.5±4.60	28.7±4.94 (↑11)
Week 78 Relative (to BW) Liver	3.17±0.41	3.08±0.55	3.45±0.77	3.76±0.48 (↑19)
Week 78 Absolute Kidney (g)	4.56±0.628	4.75±0.525	6.87±4.354	4.57±0.593
Week 78 Relative (to BW) Kidney	0.57±0.08	0.63±0.09 (↑11)	0.92±0.57* (↑61)	0.60±0.07 (↑5)
Week 78 Absolute Thyroid (mg)	35.9±11.20	35.8±8.51	50.0±12.09 (↑39)	44.5±8.48 (↑24)
Week 78 Relative (to BW) Thyroid	4.36±0.76	4.68±1.03 (↑7)	6.71±1.56** (↑54)	5.89±1.21* (↑35)
Week 104 BW (g)	771±81.7	785±129.0	748±97.3	722±108.4 (↓6)
Week 104 Absolute Liver (g)	25.9 ±5.82	25.9±7.53	25.6±3.84	27.7±4.83 (↑7)
Week 104 Relative (to BW) Liver	3.38±0.74	3.29±0.67	3.48±0.69	3.86±0.68 (↑14)
Week 104 Absolute Kidney (g)	5.16±0.693	5.60±1.194	5.83±2.337	5.41±0.846
Week 104 Relative (to BW) Kidney	0.68±0.11	0.72±0.15 (↑9)	0.77±0.22 (↑13)	0.76±0.15 (↑12)
Week 104 Absolute Thyroid (mg)	61.6±68.37 [b]	45.1±16.60 (↓27)	49.9±14.05 (↓19)	51.7±17.95 (↓16)
Week 104 Relative (to BW) Thyroid	7.96±8.50 [b]	5.80±2.11 (↓27)	6.65±1.42 (↓16)	7.17±2.21 (↓10)
Females				
Week 26 BW (g)	321±33.4	357±50.4	301±22.0	298±28.7 (↓7)
Week 26 Absolute Liver (g)	11.9±1.48	13.6±3.30	11.4±1.46	12.8±1.44 (↑7)
Week 26 Relative (to BW) Liver	3.70±0.25	3.78±0.54	3.77±0.31	4.28±0.29** (↑16)
Week 26 Absolute Kidney (g)	2.27±0.244	2.50±0.211	2.25±0.222	2.30±0.238
Week 26 Relative (to BW) Kidney	0.71±0.05	0.71±0.08	0.75±0.04	0.77±0.07 (↑8)
Week 52 BW (g)	379±39.2	396±44.4	391±44.6	345±51.1 (↓9)
Week 52 Absolute Liver (g)	14.7±2.74	14.0±2.07	12.8±1.90	14.3±1.59
Week 52 Relative (to BW) Liver	3.93±1.08	3.53±0.30	3.27±0.37	4.19±0.47 (↑7)
Week 52 Absolute Kidney (g)	2.38	2.82(↑18)	2.72 (↑14)	2.62 (↑10)
Week 52 Relative (to BW) Kidney	0.63±0.05	0.71±0.04 (↑13)	0.70±0.10 (↑11)	0.77±0.10** (↑22)
Week 78 BW (g)	510±104.9	490±75.0	439±97.0	408±76.1 (↓20)
Week 78 Absolute Liver (g)	18.4±4.35	17.3±5.95	14.3±2.84	17.1±2.65
Week 78 Relative (to BW) Liver	3.62±0.53	3.47±0.84	3.29±0.36	4.24±0.49 (↑17)
Week 78 Absolute Kidney (g)	2.92±0.218	2.99±0.522	2.85±0.504	3.21±0.623
Week 78 Relative (to BW) Kidney	0.59±0.12	0.61±0.05	0.67±0.13 (↑14)	0.79±0.012** (↑34)
Week 104 BW (g)	466±102.8	526±72.3	509±62.2	440±82.6 (↓6)
Week 104 Absolute Liver (g)	17.2±3.95	19.3±3.74	19.0±3.93 (↑10)	19.1±3.68 (↑11)
Week 104 Relative (to BW) Liver	3.70±0.47	3.66±0.54	3.75±0.74	4.39±0.64** (↑19)
Week 104 Absolute Kidney (g)	3.26±0.354	3.50±0.395	3.63±0.809 (↑11)	3.80±1.152 (↑17)
Week 104 Relative (to BW) Kidney	0.73±0.18	0.68±0.12	0.72±0.17	0.90±0.36 (↑23)

a Data were obtained from Table 10 on pages 94-101 and Appendix 8 on pages 563-572 of MRID 49575317. Percent difference from control is included in parentheses (calculated by reviewers).

b Individual thyroid wt for animal #39 reported as 337.6 mg; recalculated values without this value are absolute wt of 46.3±14.9 mg and relative (to BW) wt of 6.05±1.88.

In the kidney, progressive glomerulonephrosis was noted with slightly higher incidences in the main study 25 ppm females and the 400-ppm male rats, and was significantly increased ($p<0.05$) in the main study 1600 ppm male and female rats (23/50 treated vs. 13/50 control and 21/50 treated vs. 11/50 control, respectively) (Table 4). The incidences of this common age-related effect were all within the historical control range, and thus are not considered adverse.

The incidences of thyroid follicular cell hyperplasia and follicular cell cystic hyperplasia were increased slightly for main 1600 ppm male rats relative to control, but the incidences were generally comparable with the historical control range (Table 4). Follicular cell hyperplasia was observed in two males that also presented with follicular cell adenoma, and follicular cell hyperplasia alone was observed in only two other males of this group. Incidences of follicular cell hypertrophy were noted in 4/10 satellite males at Week 26, with no further treatment-related thyroid effects noted at Weeks 52 or 78 in the satellite rats. Thyroid findings were not observed in any treated females.

There were no adverse, treatment-related effects in the 25 ppm animals.

TABLE 4. Selected non-neoplastic microscopic findings in rats administered pethoxamid in the diet for up to 105 weeks. [a]

Observation	Concentration (ppm)			
	0	25	400	1600
Males (n=50)				
Liver				
Centrilobular hepatocyte hypertrophy	0	0	0	11**
Concentric intracytoplasmic inclusions (mainly periportal hepatocytes)	0	0	0	10**[b]
Cystic degeneration	12	5	14	24*
Clear cell hepatocytes	6	6	6	15*
Thyroid				
Follicular cell hyperplasia	0	0	1	4
Follicular cell cystic hyperplasia	4	1	4	7
Kidneys				
Progressive glomerulonephrosis	13	12	17	23*
Females (n=50)				
Liver				
Centrilobular hepatocyte hypertrophy	0	0	0	8**
Kidneys				
Progressive glomerulonephrosis	11	16	11	21*

a Data were obtained from pages 38-40 of MRID 49575317.
b Severity: trace to minimal
* Significantly different ($p<0.05$) from control; Fisher's Exact Test.
** Significantly different ($p<0.01$) from control; Fisher's Exact Test.

4. Adequacy of Dosing for Assessment of Carcinogenic Potential

Dosing up to 1600 ppm in male and female rats was considered adequate and not excessive to assess the carcinogenic potential of pethoxamid. There was a statistically significant decrease in mortality at the low dose only in males when compared to control and no statistically significant changes in mortality in females. Statistically significant non-neoplastic microscopic changes considered adverse, including thyroid enlargement, thyroid hyperplasia, cystic degeneration and clear cell hepatocytes were observed only at the highest dose in males.

B. Carcinogenicity Study in Mice

Citation: Waterson, L.A. (2000) TKC-94: Carcinogenicity study by administration to CD-1 mice for at least 80 weeks. Huntingdon Life Sciences, Ltd., Alconbury, Huntingdon, Cambridgeshire, England. Laboratory Study No.: TON 014/973848, July 10, 2000. MRID 49575318. Unpublished.

Offer, J.M. (2001) Carcinogenicity study by administration to CD-1 mice for at least 80 weeks: photomicrographic report. Huntingdon Life Sciences, Ltd., Alconbury, Huntingdon, Cambridgeshire, England. Laboratory Study No.: TON 082/000175, January 15, 2001. MRID 49813570. Unpublished.

Experimental Design:

In a carcinogenicity study (MRID 49575318), groups of 60 CD-1 (Crl:CD-1 BR) mice/sex/dose group were administered pethoxamid (TKC-94; 94.8-95.0% a.i.; Lots TB-960306 and TB-960306C) in the diet at dose levels of 0, 30, 400, or 5000 ppm (equivalent to 0/0, 4.0/5.0, 56.8/68.0, and 982/1068 mg/kg/day in males/females) for up to 95 weeks in males and 92 weeks in females. Interim sacrifice groups of 10 mice/sex/dose group were euthanized at 52 weeks.

1. Survival analysis:

There was a statistically significant negative trend for mortality in the control male mice for mortality, with $p < 0.05$ (Table 5). No statistically significant changes in mortality were observed in female mice (L. Brunsman, 9/26/2018, TXR 0057789).

Table 5. Pethoxamid – Crl:CD-1 BR Mouse Study (MRID No. 49575318)
Male Mortality Rates[+] and Cox or Generalized K/W Test Results

	Weeks					
Dose (ppm)	1-26	27-52	52[i]	53-78	79-96[f]	Total
0	0/60	5/60	8/55	7/47	13/40	25/52[*n] (48)
30	1/59[#]	7/58	7/51	11/44	6/33	25/52 (48)
400	1/60	4/59	10/55	11/45	9/34	25/50 (50)
5000	0/60	4/60	9/56	6/47	7/41	17/51 (33)

+Number of animals that died during the interval/Number of animals alive at the beginning of the interval.
[#]Animal #417 has been excluded from the analyses because the time of death reported in the individual animal histopathology appears to be incorrect, but there was not time to contact the registrant and receive correct time of death information before the CARC meeting. However, the omission of this animal does not affect the tumor analysis (see Table 6)
[i]Interim sacrifice at week 52.
[f]Final sacrifice at weeks 94-96.
[n]Negative trend.

2. Tumor Analyses:

Male mice had statistically significant trends, and statistically significant pair-wise comparisons of the 5000-ppm dose group with the controls, for liver adenomas and combined adenomas and carcinomas, all at $p < 0.01$; however, these were within the historical control range. The statistical analyses of the tumors in the male mouse study were based upon Peto's Prevalence Test (Table 6) (L. Brunsman, 9/26/2018, TXR 0057789). No statistically significant increases in tumors were observed in females up to the highest dose tested (5000 ppm).

Table 6. Pethoxamid – Crl:CD-1 BR Mouse Study (MRID No. 49575318)
Male Liver Tumor Rates[+] and Peto's Prevalence Test Results

	Dose (ppm)				
	0	30	400	5000	Historical control^
Adenomas (%)	19/46 (41)	15/41 (37)	18/42 (43)	34[a]/46 (74)	6-42%
P =	0.00009**	0.60497	0.35771	0.00073**	
Carcinomas (%)	3/43 (7)	3/33# (9)	4[b]/38 (11)	6/43 (14)	0-22%
P =	0.15490	0.41461	0.29643	0.10659	
Combined (%)	21[c]/46 (46)	16[d]/41 (39)	18[e]/42 (43)	36[e]/46 (78)	
P =	0.00004**	0.68773	0.52336	0.00059**	

+Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.
^Studies starting between 1994 and 2005
#Animal #417 was excluded from this total count. When animal #417 is included, the count is 3/34 and the P= 0.5442.
[a]First adenoma observed at week 63 in the 5000 ppm dose group.
[b]First carcinoma observed at week 75 in the 400 ppm dose group.
[c]One animal in the control group had both an adenoma and a carcinoma.
[d]Two animals in the 30 ppm dose group had both an adenoma and a carcinoma.
[e]Four animals in the each of the 400 and 5000 ppm dose groups had both an adenoma and a carcinoma.

Interim sacrifice animals have been excluded from this analysis. Adenoma counts for interim sacrifice animals were 1, 2, 0, 0 for the 0, 30, 400 and 5000 ppm dose groups, respectively. No carcinomas were observed in any interim sacrifice animals.

Animal #417 has been excluded from the analyses because the time of death reported in the individual animal histopathology appears to be incorrect (death reported on week 115 but final sacrifices took place on week 96). However, there were no tumors observed in this animal.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

3. Non-neoplastic Lesions and Other Findings:

In the interim study, terminal body weights were decreased by 26% in the males and by 23% in the females at the highest dose tested. Main study terminal body weights were decreased by 16% in the males and by 15% in the females at the highest dose tested.

At 5000 ppm in the main study, slight to marked, generalized hepatocyte hypertrophy was observed ($p<0.001$) in 39/50 males, and slight to moderate, periportal hepatocyte hypertrophy was noted ($p<0.001$) in 42/50 females, both compared to 0/50 controls. Similar findings were seen in the interim study. Moderate, generalized hepatocyte hypertrophy was observed ($p<0.001$) in 9/9 males at 5000 ppm; slight to moderate, periportal hepatocyte hypertrophy was noted in 2/10 females (not statistically significant [NS]) at 400 ppm and in 9/10 females ($p<0.001$) at 5000 ppm (Table 8).

TABLE 7a. Selected mean (±SD) body weights and body weight gains (g) in mice treated with pethoxamid via the diet for up to 92/95 weeks. [a]

Week	Dose level (ppm)			
	0	**30**	**400**	**5000**
Males				
0	32±2.3	31±2.2	31±2.5	30±2.4
13	46±4.5	46±4.6	44±4.5	39±3.5** (↓15)
26	51±6.0	51±5.7	49±6.2	42±3.7** (↓18)
39	54±6.9	53±5.9	51±6.7	43±4.2** (↓20)
52	55±6.9	54±6.3	53±7.4	44±4.6** (↓20)
78	52±6.7	54±6.8	53±6.5	43±3.7** (↓17)
95	50±6.6	53±7.1	51±5.5	42±3.1** (↓16)
BWG Weeks 0-13 [b]	14	15	13	9 (↓36)
BWG Weeks 0-26 [b]	19	20	18	12 (↓37)
BWG Weeks 0-52 [b]	23	23	22	14 (↓39)
BWG Weeks 0-78 [b]	20	23	22	13 (↓35)
BWG Weeks 0-95	19.1±6.24	22.2±7.25	19.2±5.70	11.5±3.12** (↓40)
Females				
0	24±1.9	25±1.8	25±1.8	24±1.8
13	32±4.8	32±4.5	33±3.5	29±3.1** (↓9)
26	36±7.8	37±6.1	37±5.3	32±3.4** (↓11)
39	39±9.9	39±7.2	40±6.7	33±3.8** (↓15)
52	42±12.1	40±8.1	41±7.5	34±4.3** (↓19)
78	40±7.9	42±9.0	42±8.6	35±3.9* (↓13)
92	41±7.8	42±10.6	44±8.5	35±3.6 (↓15)
BWG Weeks 0-13 [b]	8	7	8	5 (↓38)
BWG Weeks 0-26 [b]	12	12	12	8 (↓33)
BWG Weeks 0-52 [b]	18	15	16	10 (↓44)
BWG Weeks 0-78 [b]	16	17	17	11 (↓31)
BWG Weeks 0-92	17.2±7.32	18.0±9.60	19.2±8.48	11.3±3.20** (↓34)

a Data were obtained from Table 2 on pages 44-47 and Appendix 1 on pages 272-383 of MRID 49575318. Standard deviations were calculated by the reviewers. Percent differences from control (calculated by the reviewers) are included in parentheses.
b Calculated by the reviewers from data presented in this table.
* Significantly different from control; $p<0.05$
** Significantly different from control; $p<0.01$

TABLE 7b. Selected mean (±SD) absolute and relative (to body) organ weights in mice treated with pethoxamid via the diet for up to 92/95 weeks. [a]

Parameter	Dose level (ppm)			
	0	30	400	5000
Males				
Terminal body weight (g)	49.2±6.97	51.8±7.27	49.4±5.64	41.3±3.45** (↓16)
Absolute thyroid weight (mg)	7.0±1.87	6.7±1.71	7.2±2.14	8.5±2.54* (↑21)
Relative (to body) thyroid weight	0.015±0.004	0.013±0.003	0.015±0.005	0.021±0.006** (↑40)
Absolute liver weight (g)	2.95±1.225	3.27±1.339 (↑11)	3.41±1.500 (↑16)	4.89±2.550 (↑66)
Relative (to body) liver weight	6.030±2.527	6.415±2.865 (↑6)	7.018±3.284 (↑16)	11.772±5.863** (↑95)
Absolute adrenal weight (mg)	7.2±2.87	7.3±2.66	7.3±2.41	8.1±2.49 (↑13)
Relative (to body) adrenal weight	0.015±0.005	0.014±0.004	0.015±0.006	0.020±0.006** (↑33)
Females				
Terminal body weight (g)	41.4±8.39	42.6±10.85	44.1±8.95	35.2±4.16* (↓15)
Absolute thyroid weight (mg)	7.8±2.13	8.0±3.45	7.9±2.69	9.7±2.64 (↑24)
Relative (to body) thyroid weight	0.019±0.006	0.019±0.008	0.018±0.007	0.028±0.007** (↑47)
Absolute liver weight (g)	2.03±0.467	2.09±0.447	2.35±0.545 (↑16)	2.52±0.429 (↑24)
Relative (to body) liver weight	4.929±0.728	4.999±0.700	5.401±1.077 (↑10)	7.146±0.737** (↑45)
Absolute kidney weight (g)	0.515±0.073	0.535±0.101	0.592±0.091 (↑15)	0.590±0.095 (↑15)
Relative (to body) kidney weight	1.268±0.185	1.281±0.181	1.376±0.247 (↑9)	1.677±0.173** (↑32)
Absolute ovary weight (mg)	1143.9±1795.6	712.9±817.2	901.3±1614.3	554.±644.70 (↓52)
Relative (to body) ovary weight	2.880±4.783	1.683±1.884	2.132±4.011	1.526±1.613 (↓47)

a Data were obtained from Table 6 on pages 64-65 and Appendix 3 on pages 463-466 of MRID 49575318. Mean (±SD) relative (to body) organ weights were calculated by the reviewers. Percent differences from control (calculated by the reviewers) are included in parentheses.

* Significantly different from the control; $p < 0.05$.

** Significantly different from the control; $p < 0.01$.

Multiple microscopic findings were noted in the kidneys of the main study mice at the highest dose tested. However, these effects were minimal to slight, and high incidences were observed in the controls. Therefore, these effects are not considered adverse. At 5000 ppm in the main study, swelling/rarefaction of the villous epithelium of the duodenum was observed in 42/49 males and 18/49 females, and was associated with slight villous hypertrophy in 27/49 males and 5/49 females. Additionally, swelling/rarefaction of the villous epithelium of the jejunum was observed in 35/49 males and 14/49 females, and was associated with slight villous hypertrophy in 16/49 males and 2/49 females. These findings are continuations of similar observations in the interim study.

TABLE 8. Incidence of selected microscopic findings in mice treated with pethoxamid via the diet for up to 92/95 weeks. [a]						
Parameter			**Dose level (ppm)**			
			0	**30**	**400**	**5000**
Males						
Liver	Hepatocyte hypertrophy, generalized	Total	0/50	0/50	0/50	39/50***
		Slight	0/50	0/50	0/50	1/50
		Moderate	0/50	0/50	0/50	37/50
		Marked	0/50	0/50	0/50	1/50
Duodenum	Swelling/rarefaction of villous epithelium	Total	0/48	6/47*	29/47***	42/49***
	Villous hypertrophy	Slight	0/48	0/47	9/47**	27/49***
Jejunum	Swelling/rarefaction of villous epithelium	Total	0/48	4/47	25/48***	35/49***
	Villous hypertrophy	Slight	0/48	0/47	8/48**	16/49***
Kidney	Cortical tubular cell hypertrophy	Slight	0/50	0/50	0/50	8/50**
	Cortical tubules, basophilic	Total	33/50	26/50	35/50	43/50*
		Minimal	19/50	14/50	10/50	4/50
		Slight	14/50	10/50	21/50	18/50
		Moderate	0/50	2/50	4/50	21/50
Females						
Liver	Hepatocyte hypertrophy, periportal	Total	0/50	0/50	0/50	42/50***
		Slight	0/50	0/50	0/50	3/50
		Moderate	0/50	0/50	0/50	39/50
Duodenum	Swelling/rarefaction of villous epithelium	Total	0/45	3/50	12/50***	18/49***
	Villous hypertrophy	Slight	0/45	0/50	0/50	5/49
Jejunum	Swelling/rarefaction of villous epithelium	Total	0/46	2/50	7/50*	14/49***
	Villous hypertrophy	Slight	0/46	0/50	0/50	2/49
Kidney	Cortical tubules, basophilic	Total	12/50	13/50	14/50	41/50***
		Minimal	8/50	8/50	10/50	23/50
		Slight	4/50	3/50	4/50	18/50
		Moderate	0/50	2/50	0/50	0/50
	Medullary tubules dilated with eosinophilic casts	Total	14/50	14/50	13/50	32/50***
		Minimal	10/50	12/50	11/50	22/50
		Slight	4/50	2/50	2/50	10/50
	Cortical mineralization	Total	3/50	0/50	1/50	26/50***
		Minimal	2/50	0/50	0/50	22/50
		Slight	1/50	0/50	1/50	4/50
	Medullary mineralization	Total	0/50	0/50	0/50	31/50***
		Minimal	0/50	0/50	0/50	27/50
		Slight	0/50	0/50	0/50	4/50
	Papillary mineralization	Total	3/50	6/50	1/50	30/50***
		Minimal	3/50	6/50	1/50	28/50
		Slight	0/50	0/50	0/50	2/50

a Data were obtained from pages 29-33 of MRID 49575318.
* Significantly different from control; $p<0.05$
** Significantly different from control; $p<0.01$
*** Significantly different from control; $p<0.001$

4. Adequacy of Dosing for Assessment of Carcinogenic Potential

Dosing at 5000 ppm (limit dose) in male and female mice was considered adequate and not excessive to assess the carcinogenic potential of pethoxamid. There was a statistically significant negative trend for mortality in the control male mice and no statistically significant changes in mortality in female mice. Adverse non-neoplastic microscopic findings (swelling/rarefaction of the duodenum and jejunum) and changes in body weight were observed at the highest dose tested (5000 ppm) in both male and female mice.

III. TOXICOLOGY

A. Metabolism

Following single oral administration, peak plasma concentrations (t_{max}) occurred at 12 hours and similar plasma terminal half-lives were observed (43.7-46.7 hours for the 8 mg/kg group and 41-45.2 hours for the 300 mg/kg group) independently of dose or sex.

Tissue distribution was similar between males and females. The highest radioactivity concentrations occurred in whole blood, plasma, liver and kidneys in the 8 mg/kg dose group and in kidney, liver, lung, plasma and whole blood in the 300 mg/kg dose group.

The feces were the primary route of elimination for both the 8 mg/kg and 300 mg/kg dose groups, with no sex differences observed. Up to 42% and 63% of the administered dose was recovered in the feces after 24 and 96 hours, respectively. On the other hand, up to 32% and 39% of the dose was recovered in urine after 24 and 96 hours, respectively. Based on a study with bile duct-cannulated rats, the extent of absorption (the sum of the values for urine, bile, and carcass) was around 85% of the dose.

Up to eleven urinary metabolites, ten biliary metabolites (similar to the urinary metabolites), and thirteen metabolites in fecal extracts were identified, as well as the parent compound. No individual metabolites in urine or fecal extracts accounted for more than 10% of the applied dose. Unchanged parent accounted for up to 11% of the applied dose. Pethoxamid was metabolized by glutathione-S-transferase to give a number of methylthio metabolites which are further oxidized to sulphoxides and sulphones. Metabolism also occurred by cleavage of the N-(2-ethoxyethyl) group and by oxidation of methyl groups attached to the ethylenic bond. No metabolites derived from oxidation of the benzene ring were detected at all, in contrast to other well-known chloroacetamide herbicides.

B. Mutagenicity

There is no concern for mutagenic activity with pethoxamid. There was no evidence of induced mutant colonies in *in vitro* bacterial and mammalian cell assays; also, there was no evidence of unscheduled DNA synthesis in mammalian cells. Although there was evidence of chromosomal aberrations *in vitro*, the concern is lessened because no aberrations were observed in the *in vivo* mammalian micronucleus test (Table 9).

Table 9. Genotoxicity studies available for pethoxamid			
870.5100	*In vitro* Bacterial Gene Mutation (93.5% ai)	49813576 (2012) Acceptable/guideline Strains: TA98, TA100, TA1535, and TA1537 or WP2 uvrA 0, 3.16, 10.0, 31.6, 100, 316, 1000, 2500, and 5000 µg/plate in the presence and absence of S9 activation	There were no marked increases in the mean number of revertants/plate in any strain in either trial (±S9). The positive controls induced the appropriate response in all strains in the presence and absence of S9 activation. **There was no evidence of induced mutant colonies over background up to the limit dose.**
870.5100	*In vitro* Bacterial Gene Mutation (95% ai)	49813577 (1994) Acceptable/guideline Strains: TA98, TA100, TA1535, and TA1537 0, 50, 150, 500, 1500, or 5000 µg/plate in the presence and absence of S9 activation	Evidence of cytotoxicity (thinning of background lawn) was observed at 5000 µg/plate in both trials (+S9 in Trial 1 and +/−S9 in Trial 2). There were no marked increases in the mean number of revertants/plate in any strain in either trial (+/−S9). The positive controls induced the appropriate response in all strains in the presence and absence of S9 activation. **There was no evidence of induced mutant colonies over background.**
870.5300	*In vitro* Mammalian Cell Gene Mutation Assay in Chinese Hamster Lung Fibroblast (V79) Cells	49813582 (1992) Acceptable/guideline Treatment for 2 h at concentrations of 0, 10, 30, 100, 200, or 300 µg/mL (+S9) in two trials and for 24 h at concentrations of 0, 1, 3, 10, 20, or 30 µg/mL (−S9) in two trials.	No marked increase in mutant frequency was observed at any concentration in the presence or absence of S9 activation in either trial. The positive controls induced the appropriate response in the presence and absence of S9. **There was no evidence of induced mutant colonies over background in the presence or absence of S9 activation.**
870.5300	*In vitro* mammalian cell gene mutation assay (thymidine kinase locus $TK^{+/-}$) in mouse lymphoma L5178Y cells	49813586 (2015) Acceptable/guideline 0-350 µM (Trial 1, −S9, 4-h exposure); 0-450 µM (Trial 1, +S9, 4-h exposure); 0-100 µM (Trial 2, −S9, 24-h exposure); and 0-390 µM (Trial 2, +S9, 4-h exposure).	Cytotoxicity (≤20% relative total growth) was observed at the highest doses tested. No precipitation was observed at any concentration (±S9) in either trial. No biologically-relevant increases in mutant frequency were observed at any concentration in the presence or absence of S9 activation in either trial. The positive controls induced the appropriate response in the presence and absence of S9. **There was no evidence of induced mutant colonies over background in the presence or absence of S9 activation.**
870.5375	*In vitro* Mammalian Chromosome Aberration Test in Human Lymphocytes	49813584 (1994) Acceptable/guideline 0-1000 µg/mL (Trial 1; ±S9); 0-200 µg/mL (Trial 2, +S9); or 0-150 µg/mL (Trial 2, −S9).	The positive controls induced the appropriate responses in the presence and absence of S9 in both trials. **There was evidence of chromosome aberrations (clastogenic activity) induced over background in the presence and absence of S9 activation at doses that were not cytotoxic.**

Table 9. Genotoxicity studies available for pethoxamid			
870.5395	*In Vivo* Mammalian Erythrocyte Micronucleus Test (Mice)	49813591 (1994) Acceptable/guideline 0, 320, 640, or 1280 mg/kg	No decreases in polychromatic erythrocyte to normochromatic erythrocyte ratios (PCE:NCE) were observed, indicating that the test material was not toxic to the bone marrow. The positive control induced the appropriate response. **There was no increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any treatment time. Adverse signs of neurotoxicity (piloerection, hunched posture, lethargy and ptosis) and death were observed in the 1280 mg/kg dose group.**
870.5550	Unscheduled DNA Synthesis in Primary Rat Hepatocytes/Mammalian Cell Cultures	49813595 (1994) Acceptable/guideline 0, 600, or 2000 mg/kg	There was no evidence that unscheduled DNA synthesis, as determined by radioactive tracer procedures [nuclear silver grain counts], was induced.

C. Structure Activity Relationship

A search using ChemID plus (https://chem.nlm.nih.gov/chemidplus/) did not identify any relevant pesticides structurally-related to pethoxamid.

D. Sub-Chronic and Chronic Toxicity Studies

a) Subchronic toxicity study in rats

In a subchronic oral toxicity study (MRID 49575314), groups of ten CRL:CD BR rats/sex/dose were administered pethoxamid (NSK-68; 95.2% a.i.; Batch No. TB-930727) via the diet at dose levels of 0, 100, 500, 2500, or 5000 ppm (equivalent to 0/0, 7.5/8.0, 36.2/41.6, 196/207, or 388/426 mg/kg/day in males/females) for up to 13 weeks.

There were no effects of treatment on mortality, ophthalmology, or gross pathology. Body weights were decreased by 5% starting at Week 1, with continuing decreases throughout the treatment period in the 500-ppm group; cumulative overall (Weeks 0-13) body weight gains decreased by 12%. Also starting at Week 1, food consumption was decreased by 14%, with continued variable decreases throughout the treatment period. Overall food consumption was decreased by 10%. Additionally, at 500 ppm, decreased water consumption was observed in both sexes (↓14% and ↓11% in males and females, respectively) during Week 12. In the hepatic microsomal fraction evaluations, cytochrome P450 concentrations and lauric acid 11-hydroxylase and UDPGT activities were increased in females only, and activities of 7-ethoxyresorufin *O*-deethylase and 7-pentoxyresorufin *O*-dependentylase were increased in both sexes.

At 2500 ppm, body weights were decreased by 13% in the males and by 2% in the females starting at Week 1, with decreases continuing throughout the treatment period; overall body

weight gains were also decreased by 24% and 15% in males and females, respectively. Also starting at Week 1, food consumption was decreased by 23% in the males, with continued variable decreases throughout the treatment period. Overall food consumption was decreased by 9% in the males and by 7% in the females. Overall food efficiency was decreased in males as indicated by an increase in the food conversion ratio (↑19%) during Weeks 1-13. During Week 12, decreased water consumption was observed in both sexes (↓20% and ↓13% in males and females, respectively). Increases were noted in cholesterol levels (↑46% and ↑37% in males and females, respectively) and total protein concentrations (↑5% and ↑4% in males and females, respectively). Absolute liver weight (↑7% and ↑15% in males and females, respectively) and relative (to body) liver weights (↑27% and ↑22% in males and females, respectively) were increased in animals at 2500 ppm. Absolute thyroid weight was increased in males at ≥2500 ppm (↑15-17%) and in females at all doses (↑16-24%). Relative (to body) thyroid weights were increased in males and females (↑39% and ↑22%, respectively) at 2500 ppm. Microscopically, periportal hepatocytes with margination of cytoplasm were observed in both sexes (males>females). Occasional concentric intracytoplasmic inclusions were observed in periportal hepatocytes in males only. An increased incidence of periportal hepatocyte fat deposition was observed in males only. In the thyroid, the incidence of follicular cell hypertrophy was increased in males only. In the hepatic microsomal fractions, protein, cytochrome P450 concentrations, and 7-ethoxyresorufin *O*-deethylase, 7-pentoxyresorufin *O*-dependentylase, lauric acid 11-hydroxylase, and UDPGT activities were increased in both sexes.

At 5000 ppm, body weights were decreased by 25% in the males and by 13% in the females starting at Week 1, with decreases continuing throughout the treatment period; overall body weight gains were decreased in both sexes by ↓31% and ↓25% in males and females, respectively. Starting at Week 1, decreases in food consumption were observed in both sexes (↓49% and ↓24% in males and females, respectively) with continued variable decreases throughout the treatment period. Overall food consumption was decreased in both sexes (↓15% and ↓10% in males and females, respectively). Overall food efficiency was decreased in both sexes as indicated by an increase in the food conversion ratio from Weeks 1-13 (↑23% and ↑19% in males and females, respectively). During Week 12, decreased water consumption was observed in both sexes (both ↓16%). Increases were observed in cholesterol levels (↑108% and ↑75% in males and females, respectively). Glucose values were decreased in both sexes (↓13% and ↓11% in males and females, respectively). Absolute liver weights were increased (↑16% and ↑29% in males and females, respectively) and relative (to body) liver weights were increased (↑41% and ↑38% in males and females, respectively) compared to control values. Relative (to body) thyroid weights were increased in males and females at 5000 ppm (↑41% and ↑38%). Microscopically, increased incidences of periportal hepatocytes with margination of cytoplasm, occasional concentric intracytoplasmic inclusions in periportal hepatocytes, and minimal generalized hepatocyte enlargement were observed in both sexes. An increased incidence of periportal hepatocyte fat deposition was observed in the males. In the thyroid, the incidence of follicular cell hypertrophy was increased in the males, and an increased incidence of thyroids with sparse colloid was observed in both sexes. In the hepatic microsomal fractions, protein, cytochrome P450, and 7-ethoxyresorufin *O*-deethylase, 7-pentoxyresorufin *O*-dependentylase, lauric acid 11-hydroxylase, and UDPGT activities were increased in both sexes.

b) Subchronic toxicity study in mice

In a subchronic oral toxicity study (MRID 49575316), groups of ten CRL:CD1-1 (ICR) BR mice/sex/dose were administered pethoxamid (TKC-94; 95.0% a.i.; Lot TB-960306) via the diet at dose levels of 0, 50, 400, 3000, or 10,000 ppm (equivalent to 0/0, 9.1/12, 70.5/93, 610/724, or 2354/2492 mg/kg/day in males/females) for up to 13 weeks.

There were no effects of treatment on clinical signs, mortality, food consumption, or ophthalmology.

Body weights were decreased by 9% in the males and by 4% in the females beginning at Week 1 in the 3000-ppm group, with decreases continuing throughout the treatment period for females (up to 13% by week 12). Body weight gains were decreased by 70% in the males and by 75% in the females during Weeks 0-1, and by 42% in the females for the duration of the treatment period. Mean food conversion ratios were higher in females (↑56%) from Weeks 2-12. This is supportive of treatment-related decreases in body weight gains.

Mean albumin concentrations were decreased by 10% in the males and by 6% in the females; and cholesterol levels were increased by 33% in the males and by 74% in the females in the 3000-ppm group. Body weight-adjusted liver weight was increased in both sexes (↑26% and ↑22% in males and females, respectively) compared to control values. Increased incidence/severity of hepatocyte hypertrophy (generalized/centrilobular and midzonal in males and periportal in females) was observed that correlated with increased liver weights and cholesterol values in animals at this dose.

Decreased body weights were observed in both sexes (↓15% and ↓8% in males and females, respectively) beginning at Week 1 in the 10,000-ppm group, with decreases continuing throughout the treatment period (up to 23% in males and 19% in females by week 12). Body weight losses were observed in the males (−2.7 g treated vs/ +2.3 g control) and females (−1.1 g treated vs. +1.2 g control) during Weeks 0 to 1, with a loss (−0.2 g treated vs. +9.9 g control) for Weeks 0-12 noted in the males, and an 80% decrease noted for Weeks 0-12 in the females. Mean food conversion ratios were higher in both sexes (↑212% and ↑138% in males and female, respectively) from Weeks 2 12.

At 10,000 ppm, decreases in hematocrit (↓8% and↓7% in males and females, respectively), hemoglobin (↓7% and ↓8% in males and females, respectively), and red blood cell count (↓12% and ↓8% in males and females, respectively) were observed compared to control values. Decreases in mean corpuscular hemoglobin count (↓3%), white blood cell count (↓34%), and lymphocyte count (↓41%) were observed in males only. Total protein concentration was decreased in both sexes (↓11% and ↓6% in males and females, respectively) which was associated with decreased albumin levels in both sexes (↓10% and ↓13% in males and females, respectively) and globulin level in males only (↓12%). Cholesterol levels were increased in both sexes (↑33% and ↑108% in males and females, respectively). Calcium and potassium

concentrations were decreased (↓8% and p≤0.05, ↓16%, respectively), and chloride and phosphorus concentrations were increased in males only (↑4% and ↑15, respectively).

Urinalysis revealed lower protein concentrations in both sexes at 10,000 ppm (298 mg/dL vs. 668 mg/dL control and 49 mg/dL vs. 199 mg/dL control, respectively). Additionally, there was an increased incidence of marked ketonuria in females.

Terminal body weights were decreased at 10,000 ppm by 25% in the males. Absolute liver weights were increased at 3000 ppm (↑20% and ↑19% in males and females, respectively) and 10,000 ppm (↑21% and ↑45% in males and females, respectively) compared to control values. Relative (to body) liver weights also were increased at 3000 ppm (↑30% and ↑25% in males and females, respectively) and 10,000 ppm (↑62% and ↑53% in males and females, respectively). Absolute spleen weight was decreased in both sexes (↓33% and ↓27% in males and females, respectively). The only treatment-related gross pathological effect was a reduction in adipose tissue in males compared to control (6/10 vs. 0/10 control). Statistically significant increases in the incidence/severity of hepatocyte hypertrophy (generalized/centrilobular and midzonal in males and periportal in females) was observed at 10,000 ppm but these effects were considered adaptive. Also, swelling and cytoplasmic rarefaction of villous epithelial cells in the duodenum was observed in males and females, starting at 3000 ppm. However, these intestinal effects are only considered adverse in the presence of clinical signs (e.g. decreased body weights), which only occurred at the highest dose tested.

2. Chronic Toxicity Studies

a) Chronic/carcinogenicity toxicity study in rats

In a carcinogenicity study (MRID 49575317), groups of 80 Crl:CD BR rats/sex/dose were administered pethoxamid (TKC-94; 95.0% a.i.; Lot No. TB-960306) in the diet at concentrations of 0, 25, 400, or 1600 ppm (equivalent to 0/0, 1.0/1.4, 17.0/23.3, and 70/99 mg/kg/day in males/females) for up to 105 weeks. Interim sacrifices were conducted on ten rats/sex/dose level at Weeks 26, 52, and 78.

There were no effects of treatment on mortality/survival, clinical signs, water consumption, ophthalm3oscopic examinations, hematology or urinalysis parameters.

Main and satellite females, and satellite males that were administered 400 ppm had generally decreased mean body weights and body weight gains relative to control. There was no effect on body weights in the males in either the satellite or main study groups. In females, a non-significant 14% decrease in body weights was observed at week 78 in the satellite group and non-significant 6 to 9% decrease was seen from week 26 to 88. Body weight gains in the main group females were decreased by 14% and 11% for Weeks 4-88, and Weeks 0-88, respectively. Body weight gains in the satellite males and females were decreased by 12% and 19%, respectively, for Weeks 0-78.

Decreases in body weight gain were observed in the 1600 ppm animals throughout the treatment period (Weeks 0-104 and Weeks 0-78) for the main and satellite groups, respectively. Body weights were decreased in the main animals from 7 to 11% (males) and 5-19% in females. These findings were observed from week 2 to termination but were not significant. Body weight gains in the males and females were decreased by 13% and 23%, respectively, for Weeks 0-88, and decreased by 10% and 8%, respectively, for Weeks 0-104. Body weights were decreased in the satellite animals by 5 -11% (males commencing at week 2) and by 7-20% (females, starting at week 26). Body weight gains were decreased by 15% and 8%, respectively, for Weeks 0-4, and decreased by 9% and 28%, respectively, for Weeks 0-78.

Cumulative food consumption for Weeks 1-4 was decreased for the main 400 ppm males and females by 4% each. Food intake and cumulative consumption generally was similar to control for all 400 ppm animals after Week 4 until scheduled termination. Mean food efficiency values (up to Week 26) for the main and satellite females administered 400 ppm were increased relative to control values.

Throughout the treatment period (Weeks 1 to 104), 1600 ppm males in the main group demonstrated consistent decreases in cumulative mean food intake, with large decreases in weekly consumption during Weeks 1 and 3 (↓11%) and again during Weeks 85-103 (↓ up to 19%). Cumulative food consumption in the main group males was decreased by 8% for Weeks 1-4, and cumulative food consumption continued to be decreased by 5-10%, with an overall cumulative consumption decrease of 6%. Cumulative food consumption for Weeks 1-4 was decreased for main group females and all satellite animals by 5-6%. Food intake and cumulative consumption generally was similar to control for all main and satellite females, and satellite males, after Week 4 until scheduled termination. Mean food efficiency values (up to Week 26) for the main and satellite animals were increased relative to control values, and it was stated that these differences reflected inefficient food utilization.

In the 1600 ppm males, γGT concentrations were increased ($p \leq 0.01$) at all weeks of investigation. γGT concentrations were not different from control for 1600 ppm females. Total cholesterol concentrations were increased in all weeks of investigations in both sexes. Females also had increased mean globulin concentrations of 9-13% for Weeks 13, 26, and 52, with lesser increases for Weeks 78 and 104. Generally concomitant, but slight, increases in total protein concentrations also were noted at all weeks of investigation, with the largest increase of 10% at Week 52. There were no treatment-related effects on globulin/protein concentrations in males.

Mean relative (to body weight) liver weights were increased by 14-19% at the interim sacrifices, and at Week 104 for males. In females, mean relative (to body weight) liver weights were increased by 16-19% at Weeks 26, 78, and 104. Generally non-dose-dependent mean relative (to body weight) thyroid weight effects were apparent in treated males, but not females, at Weeks 26, 52, and 78. An apparent decrease in relative (to body weight) thyroid weights at Week 104 was abolished after removal of a single control group value that was an outlier.
At Week 104, treatment-related gross pathological findings in the 1600 ppm animals included: thyroid enlargement (12/50 treated vs. 4/50 control) and enlarged liver (6/50 treated vs. 2/50 control) in males only. A treatment-related increase in the incidence of centrilobular hepatocyte hypertrophy was seen in the main group male and females (11/50 and 8/50, respectively treated

vs. 0/50 controls). This finding was considered associated with the increased mean body weight-adjusted liver weights noted for male and females, as well as the increased incidence of liver enlargement reported macroscopically for males. Treatment-related incidences of centrilobular hepatocyte hypertrophy also were seen in satellite males and females at Week 26, and in satellite males at Weeks 52 and 78. Additionally, increased incidences of hepatocytes with concentric intracytoplasmic inclusions (mainly in periportal zones), focal cystic degeneration, and focal clear cell hepatocytes were observed in males. Increased incidences of hepatocytes with concentric intracytoplasmic inclusions (mainly in periportal zones) also were noted at Weeks 26 and 52 in the satellite males.

In the kidney, progressive glomerulonephrosis was noted with slightly higher incidences in the main study 25 ppm females and the 400-ppm male rats, and was significantly increased ($p<0.05$) in the main study 1600 ppm male and female rats (23/50 treated vs. 13/50 control and 21/50 treated vs. 11/50 control, respectively). The incidences of this common age-related effect were all within the historical control range (Appendix 1), and thus are not considered adverse.

The incidences of thyroid follicular cell hyperplasia and follicular cell cystic hyperplasia were increased slightly for main 1600 ppm male rats relative to control, but the incidences were generally comparable with the historical control range. Follicular cell hyperplasia was observed in two males that also presented with follicular cell adenoma, and follicular cell hyperplasia alone was observed in only two other males of this group. Incidences of follicular cell hypertrophy were noted in 4/10 satellite males at Week 26, with no further treatment-related thyroid effects noted at Weeks 52 or 78 in the satellite rats. Thyroid findings were not observed in any treated females.

There were no adverse, treatment-related effects in the 25 ppm animals.

b) Carcinogenicity study in mice

In a carcinogenicity study (MRID 49575318), groups of 60 CD-1 (Crl:CD-1 BR) mice/sex/dose group were administered pethoxamid (TKC-94; 94.8-95.0% a.i.; Lots TB-960306 and TB 960306C) in the diet at dose levels of 0, 30, 400, or 5000 ppm (equivalent to 0/0, 4.0/5.0, 56.8/68.0, and 982/1068 mg/kg/day in males/females) for up to 95 weeks in males and 92 weeks in females. Interim sacrifice groups of 10 mice/sex/dose group were euthanized at 52 weeks.

There were no effects of treatment on mortality or clinical signs of toxicity.

In the interim group, body weight gains in both sexes were consistently decreased throughout treatment, resulting in decreases in interim (Weeks 0-52) body weight gains and body weights in both sexes at 5000 ppm. In the main group, body weight gains in both sexes were consistently decreased throughout treatment, resulting in decreased overall body weight gains for both the males (Weeks 0-95; ↓40%) and the females (Weeks 0-92; ↓34%) at 5000 ppm. Body weights were decreased throughout the study by 13-22% in the males and by 4 19% in the females.

In the interim study, terminal body weights were decreased by 26% in the males and by 23% in the females. Absolute and adjusted kidney weights were increased by 11% and 19% in the

females. Main study terminal body weights were decreased by 16% in the males and by 15% in the females. In the females, absolute and adjusted kidney weights were increased by 23%.

At 5000 ppm in the main study, slight to marked, generalized hepatocyte hypertrophy was observed ($p<0.001$) in 39/50 males, and slight to moderate, periportal hepatocyte hypertrophy was noted ($p<0.001$) in 42/50 females, both compared to 0/50 controls. Similar findings were seen in the interim study. Moderate, generalized hepatocyte hypertrophy was observed ($p<0.001$) in 9/9 males at 5000 ppm; slight to moderate, periportal hepatocyte hypertrophy was noted in 2/10 females (NS) at 400 ppm and in 9/10 females ($p<0.001$) at 5000 ppm (table 9). Multiple microscopic findings were also noted in the kidneys of the main study mice at the highest dose tested. However, these effects were minimal to slight, and high incidences were observed in the controls. Therefore, these effects are not considered adverse.

At 5000 ppm in the main study, swelling/rarefaction of the villous epithelium of the duodenum was observed in 42/49 males and 18/49 females, and was associated with slight villous hypertrophy in 27/49 males and 5/49 females. Additionally, swelling/rarefaction of the villous epithelium of the jejunum was observed in 35/49 males and 14/49 females, and was associated with slight villous hypertrophy in 16/49 males and 2/49 females. These findings are continuations of similar observations in the interim study.

IV. MODE OF ACTION

A. Key and Associative Events

The following mode of action has been postulated by the Registrant for pethoxamid-induced rat thyroid and mouse liver tumors, which is through activation of the constitutive androstane receptor (CAR). The following information was extracted from the registrant's mode of action white paper (MRID 49813573):

The proposed MOA is composed of three key events:

Key Event 1: Activation of CAR
Key Event 2: Induction of replicative DNA synthesis
Key Event 3: Formation of liver or thyroid follicular cell adenomas.

The proposed MOA also states that CAR activation will elicit increased expression of cytochrome P450s, increased incidence of hepatocellular hypertrophy, and increased liver weight; however, these events are considered associative and thus not integral to the induction of liver tumors.

1. CAR Activation (Key Event 1)

No studies showing direct activation of the CAR receptor by pethoxamid were provided. Two studies showing the induction of cytochrome P450 expression and activity, an associative event, are available (MRID 49813526 and 49813571).

Rat

Hepatic microsomes prepared from liver tissue samples from male rats administered pethoxamid (400 or 1600 ppm) in the diet for 7 or 14 days were evaluated (MRID 49813526). At 1600 ppm, cytochrome b5 and CYP450 contents were increased by 22% and 63%, respectively ($p<0.05$), and thyroxine glucuronidation was increased by 62% ($p<0.05$). At 400 ppm, CYP450 content was increased by 17% ($p<0.05$), with no other treatment-related effects observed (Table 10).

TABLE 10. Mean (±SD) hepatic microsomal enzyme activity in male rats treated with pethoxamid in the diet for 14 days or after a 42-day recovery period. [a]

Parameter	Dose level (ppm)				
	0	400	1600	0 [b]	1600 [b]
Liver weight (g)	15.1±1.7	14.5±1.5	16.9±1.7 (↑12)	16.7±2.8	16.4±2.0
Hemoglobin content (nmol/ mg protein)	0.849±0.206	0.724±0.213	0.707±0.128	1.25±0.24	1.02±0.16
Cytochrome b5 content (nmol/ mg protein)	0.463±0.047	0.516±0.042	0.565±0.067* (↑22)	0.552±0.043	0.601±0.044* (↑9)
CYP450 content (nmol/mg protein)	0.567±0.079	0.663±0.058* (↑17)	0.923±0.0258* (↑63)	0.683±0.095	0.706±0.049
Thyroxine glucuronidation (area ratio)	0.0487±0.0078	0.0557±0.0082	0.0788±0.0075* (↑62)	0.0543±0.0125	0.0511±0.0072

a Data were obtained from Table 7.3 on page 41 of MRID 49813526; n=8/group. Percentage differences from control, calculated by the reviewers, are presented in parentheses.
b After a 42-day recovery period.
* Significantly different from control; $p<0.05$.

At 1600 ppm, UGT1A1 and 1A6 mRNA levels were increased by 23% and 278%, respectively ($p<0.05$), for male rats. At 400 ppm, the UGT1A6 mRNA level was increased by 82% ($p<0.05$), with no treatment effect observed on the UGT1A1 mRNA level (Table 11).

TABLE 11. Mean (±SD) hepatic UGT mRNA levels (fold change) in male rats treated with pethoxamid in the diet for 14 days or after a 42-day recovery period. [a]

CYP450 isozyme	Dose level (ppm)				
	0	400	1600	0 [b]	1600 [b]
UGT1A1	1.00±0.00	1.05±0.12	1.23±0.25*	1.00±0.00	1.01±0.14
UGT1A6	1.00±0.00	1.82±0.42*	3.78±0.62*	1.00±0.00	0.919±0.334

a Data were obtained from Table 7.5 on page 43 of MRID 49813526; n=8/group.
b After a 42-day recovery period.
* Significantly different from control; $p<0.05$.

Mouse

In a two-week study (MRID 49813571), groups of 18 ICR (crj:CD-1) male mice/dose level were administered pethoxamid (95.0% a.i.) in the diet at doses of 0, 30, 400, or 5000 ppm (equivalent to 0, 3.92, 49.1, or 541 mg/kg/day) for up to 14 days.

At 5000 ppm, microsomal protein content and CYP450 content were increased by 21% and 95%, respectively ($p<0.01$), and microsomal PROD activity was increased by 5567% ($p<0.01$) (Table

12). In addition, at 5000 ppm, relative increases of 329%, 745%, 114%, and 64% were noted in CYP1A, 2B, 3A2, and 4A1 content, respectively ($p<0.01$) (Table 13). No treatment-related effects in microsomal protein content and total CYP450, or individual isozyme contents, were noted for the 400 ppm animals, but microsomal PROD activity was increased by 767% ($p<0.05$). No other treatment-related effects on microsomal parameters were observed in the ≤ 400 ppm animals.

TABLE 12. Mean (±SD) hepatic microsomal enzyme activity in male mice treated with pethoxamid in the diet for 14 days. [a]

Parameter	Dose level (ppm)			
	0	30	400	5000
Microsomal protein content (mg/g liver)	42±3	44±2	45±4	51±3** (↑21)
CYP450 content (nmol/mg liver)	0.60±0.09	0.56±0.09	0.60±0.07	1.17±0.15** (↑95)
PROD activity (pmol/min/mg liver)	3±2	11±5	26±6* (↑767)	170±11** (↑5567)

a Data were obtained from Table 8 on page 42 of MRID 49813571, n=6/group. Percentage differences from control, calculated by the reviewers, are presented in parentheses.
* Significantly different from control; $p<0.05$.
** Significantly different from control; $p<0.01$.

TABLE 13. Mean (±SD) hepatic microsomal CYP450 isoenzyme content in male mice treated with pethoxamid in the diet for 14 days. [a]

CYP450 isozyme relative value (%) [b]	Dose level (ppm)			
	0	30	400	5000
CYP1A	2.94±0.36	2.38±0.54	2.37±0.21** (↓19)	12.6±1.5** (↑329)
CYP2B	1.48±0.18	1.41±0.23	1.56±0.34	12.5±1.7** (↑745)
CYP3A2	15.0±3.2	14.3±4.0	14.7±3.3	32.1±3.8** (↑114)
CYP4A1	10.0±2.2	10.0±1.7	12.0±2.0	16.4±3.4** (↑64)

a Data were obtained from Table 9 on page 43 of MRID 49813571, n=6/group. Percentage differences from control, calculated by the reviewers, are presented in parentheses.
b Ratio to standard protein.
** Significantly different from control; $p<0.01$.

In a second non-guideline induction study (MRID 49813526), liver tissues (n=8/dose level/species) from CRL:CD-1 male mice administered pethoxamid (92.6% a.i.) in the diet at doses of 0, 400, or 5000 ppm for seven days and CRL:CD male rats administered pethoxamid in the diet at doses of 0, 400, or 1600 ppm for 14 days were used in *ex vivo* investigations.

Hepatic microsomes prepared from liver tissue samples from male mice administered pethoxamid in the diet for 7 or 14 days, respectively, were evaluated. In mice, no treatment-related effects were reported for liver weights, although an increase of 23% occurred at the 5000-ppm level (NS). At 5000 ppm, cytochrome b5 and CYP450 contents were increased by 38% and 50%, respectively ($p<0.05$). In addition, CYP450 content was increased by 22% at the 400-ppm dose level ($p<0.05$) (with no effect on cytochrome b5 content) (Table 14).

TABLE 14. Mean (±SD) hepatic microsomal enzyme activity in male mice treated with pethoxamid in the diet for 7 days or after a 42-day recovery period. [a]

Parameter	Dose level (ppm)				
	0	400	5000	0 [b]	5000 [b]
Liver weight (g)	2.10±0.66	1.90±0.28	2.59±0.25 (↑23)	1.95±0.22	2.27±0.19
Hemoglobin content (nmol/ mg protein)	1.13±0.28	1.45±0.52	1.68±0.46	1.17±0.11	1.37±0.13
Cytochrome b_5 content (nmol/ mg protein)	0.507±0.092	0.535±0.068	0.702±0.063* (↑38)	0.538±0.049	0.511±0.054
CYP450 content (nmol/mg protein)	0.795±0.138	0.968±0.129* (↑22)	1.19±0.09* (↑50)	0.835±0.067	0.714±0.147
7-EROD activity (pmol/mg protein/min)	167±35	281±121* (↑68)	257±65* (↑54)	138±25	119±19
Testosterone 6β-hydroxylation (pmol/mg protein/min)	715±168	3140±640* (↑339)	906±374	616±125	522±110
Testosterone 16β-hydroxylation (pmol/mg protein/min)	246±59	359±84* (↑46)	429±195* (↑74)	272±34	221±34** (↓19)
Lauric acid 12-hydroxylation (pmol/mg protein/min)	1360±690	1260±410	1650±870	796±186	827±119

a Data were obtained from Table 7.6 on pages 44-45 of MRID 49813526; n=8/group. Percentage differences from control, calculated by the reviewers, are presented in parentheses.
b After a 42-day recovery period.
* Significantly different from control; $p<0.05$.
** Significantly different from control; $p=0.01$.

At 5000 ppm, mRNA analyses demonstrated relative increases of 97%, 11400%, 590%, and 800% for CYP1A2, 2B10, 3A11, and 4A10 mRNA levels in male mice ($p<0.01$). At 400 ppm, only an increase of 1140% for CYP2B10 was observed ($p<0.05$) (Table 15).

TABLE 15. Mean (±SD) hepatic CYP450 mRNA levels (fold change) in male mice treated with pethoxamid in the diet for 7 days or after a 42-day recovery period. [a]

CYP450 isozyme	Dose level (ppm)				
	0	400	5000	0 [b]	5000 [b]
CYP1A2	1.00±0.00	1.06±0.47	1.97±0.47*	1.00±0.00	0.685±0.299
CYP2B10	1.00±0.00	12.4±5.4*	115±32*	1.00±0.00	0.907±0.486
CYP3A11	1.00±0.00	1.21±0.57	6.90±0.90*	1.00±0.00	0.803±0.337
CYP4A10	1.00±0.00	2.21±1.96	9.00±13.60*	1.00±0.00	0.707±0.370

a Data were obtained from Table 7.8 on page 48 of MRID 49813526; n=8/group.
b After a 42-day recovery period.
* Significantly different from control; $p<0.05$.

After the 42-day recovery period, values for all examined parameters in mice and rats treated with pethoxamid at dose levels up to 5000 ppm and 1600 ppm, respectively, generally were comparable with control values. Although the data were not provided, it was stated that the experimental conditions demonstrated acceptable linearity with respect to incubation time and microsomal protein concentration, and the expected responses for enzyme-catalyzed reactions. The registrant stated that the results of these investigations demonstrate that pethoxamid behaves as a phenobarbital-type enzyme inducer at doses up to 5000 ppm (541 mg/kg/day in male mice [MRID 49813571] and 972/130 mg/kg/day in male mice/rats [MRID 49813526]). Phenobarbital is a classic CYP2B inducer, and CYP2B10 is considered the major phenobarbital-inducible

CYP450 isozyme. In MRID 49813571, treatment with pethoxamid resulted in increases in liver weight (and enlarged livers), microsomal protein and CYP450 contents, PROD activity, and CYP2B content. In addition, the pattern of early cell proliferation and decreases in hepatic gap junctional intercellular communication after pethoxamid administration were similar to phenobarbital and/or supportive of hepatic induction and growth stimulation. All effects were noted at 5000 ppm, but increased PROD activity occurred at ≥400 ppm and decreases in hepatic CX32 spots occurred at ≥30 ppm. The observed effects were considered adaptive and not adverse. In MRID 49813526, administration of pethoxamid at 5000 and 1600 ppm to male mice and rats, respectively, yielded fold-induction results in cytochrome b_5 and CYP450 contents similar to phenobarbital and other inducers (historical positive control data). In addition, administration of pethoxamid up to 5000 ppm yielded fold-induction results for CYP2B10 activity and mRNA levels similar to phenobarbital, as compared to concurrent positive control data in mice. The registrant indicated that the results of these investigations show that pethoxamid is capable of CYP450 induction with responses similar to phenobarbital.

CARC conclusions for Key Event 1 (CAR activation): Although there were no data to support direct binding of pethoxamid to CAR, the CARC agreed that the data submitted to support Key Event 1 are consistent with CAR activation in both rats and mice.

2. Induction of replicative DNA synthesis (Key Event 2)

Rat

The BrdU labeling index was measured in male rats treated with pethoxamid for 0, 400, or 1600 ppm for 14 days (MRID 49813533). An increase in thyroid DNA replication (NS), as measured by BrdU labeling, was observed in rats at 1600 ppm, but not at 400 ppm (Table 16a). These effects were reversed after a 42-day recovery period.

TABLE 16a. Mean (±SD) cellular BrdU labeling values in male mice and rats treated with pethoxamid in the diet for 7 (mice) or 14 (rats) days or after a 42-day recovery period. [a]

Parameter	Dose level (ppm)				
	Main			Recovery	
	0	400	5000 [b] / 1600 [c]	0	5000 [b] / 1600 [c]
Mice					
Number of tissues examined	8	8	8	8	8
BrdU value (Liver)	0.2±0.1	0.6±0.6	12.2±2.3*** (↑6000)	0.1±0.1	0.1±0.1
Rats					
Number of tissues examined	8	8	8	8	8
BrdU value (Thyroid)	3.0±1.8	3.0±1.1	7.2±6.1 (↑140)	0.9±0.7	1.3±0.8

a Data were obtained from Tables 10A and B on pages 83 and 85 of MRID 49813533. Percentage differences from control, calculated by the reviewers, are presented in parentheses.
b High dose for mice.
c High dose for rats.
*** Significantly different from control; $p<0.001$.

Thyroid hormone levels were also measured for rats in the same study that measured BrdU levels (MRID 49813533). The inter-subject, and within-group, variabilities for thyroid hormone levels from rats administered pethoxamid were large. Generally, mean values were comparable to control levels in the main toxicity groups, although TSH concentration was increased (NS) by 12% in the 1600 ppm group, and total T4 concentration was increased by 10% (NS) in the 400

ppm group and by 18% ($p<0.05$) in the 1600 ppm group (Table 16b). Thus, these data are considered inadequate to support the proposed thyroid MOA for rats. No treatment-related effects were noted for thyroid hormone levels after the 42-day recovery period.

TABLE 16b. Mean (±SD) serum thyroid hormone parameters in male rats treated with pethoxamid in the diet for 14 days or after a 42-day recovery period. [a]

Parameter	Dose level (ppm)				
	Main			Recovery	
	0	400	1600	0	1600
TSH (ng/mL)	5.788±3.482	5.194±2.896	6.507±3.132 (↑12)	5.345±4.132	4.927±3.373
Total T4 (µg/mL)	4.662±0.768	5.115±0.569 (↑10)	5.504±1.143* (↑18)	5.795±1.877	5.293±1.304
Free T3 (pg/mL)	3.547±0.890	3.585±0.408	3.754±0.763	4.043±1.235	3.441±0.914

a Data were obtained from Table 9 on page 81 of MRID 49813533; n=15/group. Percentage differences from control, calculated by the reviewers, are presented in parentheses.

* Significantly different from control; $p<0.05$.

Mouse

The BrdU labeling index was measured in male mice treated with pethoxamid for 0, 400, or 5000 ppm for 7 days (MRID 49813533). A significant increase in hepatic DNA replication (increased BrdU labeling index) was observed at the highest dose tested ($p<0.001$; 5000 ppm).

Induction of replicative DNA synthesis is associated with cell proliferation; thus, increased cell proliferation is considered part of this key event by the reviewer. Although not described as part of this key event by the registrant, the two-week mouse study (MRID 49813571) also assessed hepatic cell proliferation by measuring the hepatic PCNA labelling index. The PCNA labeling index was increased after pethoxamid administration at 5000 ppm for three or seven days by 277% and 681%, respectively ($p<0.05$) (Table 17). There were no treatment-related effects for any administration period on the PCNA labeling index at 30 or 400 ppm.

TABLE 17. Mean (±SD) hepatic PCNA labelling index (%) in male mice treated with pethoxamid in the diet for up to 14 days [a]

Day	Dose level (ppm)			
	0	30	400	5000
3	0.26±0.18	0.28±0.15	0.35±0.32	0.98±0.49* (↑277)
7	0.27±0.21	0.33±0.40	0.20±0.21	2.11±0.99* (↑681)
14	0.16±0.12	0.37±0.46	0.21±0.07	0.26±0.32

a Data were obtained from Table 10 on page 44 of MRID 49813571; n=6/dose level/treatment period. Percentage differences from control, calculated by the reviewers, are presented in parentheses.

* Significantly different from control; $p<0.05$.

CARC conclusions for Key Event 2 (induction of replicative DNA synthesis): the CARC agreed that the data submitted to support Key Event 2 are consistent with CAR activation in mice. However, the induction of replicative DNA synthesis is considered equivocal in rats.

Thyroid and Hepatocyte Hypertrophy (Associative Event)

Although described as a key event by the registrant, the CARC considers liver and thyroid follicular cell hypertrophy to be associative events.

Rat

In rats, there was a significant increase in thyroid follicular cell hypertrophy at 1600 ppm but not at 400 ppm in males following treatment with pethoxamid for 7 days, which was reversed after a 42-day recovery period (MRID 49813533). There was also a significant increase in thyroid follicular cell hypertrophy in a 90-day study in rats (MRID 49575314) at the highest dose tested (5000 ppm) in males (9/10 vs 2/10 in the control) and females (4/10 vs 0/10 in the control). However, no thyroid hypertrophy was observed in the chronic study in rats.

Mouse

In mice, hepatocyte hypertrophy was significantly increased in males, starting at 5000 ppm, and in females, starting at 3000 ppm, throughout the database (Table 18). Hepatocyte hypertrophy was reversed after a 42-day recovery period (MRID 49813533).

Table 18. Incidence of Hepatocellular Hypertrophy in Mice[1]

Parameter	Dose (ppm)							
	30	**50**	**400**	**3000**	**5000**	**10000**	**Duration**	**MRID**
Hepatocellular hypertrophy	**Males**							
	--	--	2/8	--	8/8**	--	7 days	49813533
	--	0/10	0/10	1/10	--	8/10**	90 days	49575316[a]
	0/8	--	0/10	--	9/9**	--	364 days	49575318
	0/50	--	0/50	--	39/50**	--	655 days	49575318
Hepatocellular hypertrophy	**Females**							
	--	--	--	--	--	--	7 days	49813533
	--	0/10	0/10	10/10**	--	10/10**	90 days	49575316[b]
	0/8	--	2/10	--	9/10**	--	364 days	49575318[b]
	0/50	--	0/50	--	42/50**	--	644 days	49575318[b]

[1]Table compiled by EPA reviewer

Statistically different from control *p<0.05; **p<0.01

[a]Centrilobular midzonal hepatocyte hypertrophy

[b]Periportal hepatocyte hypertrophy

Increased Liver and Thyroid Weight (Associative Event)

Although described as a key event by the registrant, the reviewer considers changes in organ weight an associative event. In rats, thyroid weights were significantly increased in males starting at 400 ppm and in females starting at 5000 ppm (Table 19). In mice, significant liver weight increases were observed starting at 3000 ppm in repeated-dose studies (Table 20).

Table 19. Thyroid weight changes in Rats[1]

Parameter	Dose (ppm)								
	25	**100**	**400**	**500**	**1600**	**2500**	**5000**	**Duration**	**MRID**
Thyroid weight					**Males**				
	--	--	A: ↑29%** R: ↑29%**	--	A: ↑43%** R: ↑45%**	--	--	7 days	49813533
	--	NS	--	NS	--	A: ↑17% R: ↑39%*	A: ↑15% R: ↑41%**	90 days	49575314
	NS	--	A: ↑19% R: ↑36%*	--	A: ↑31% R: ↑47%**	--	--	364 days	49575317
	NS	--	NS	--	NS	--	--	728 days	49575317
					Females				
	--	--	--	--	--	--		7 days	49813533
	--	NS	--	NS	--	A: ↑16% R: ↑22%	A: ↑24% R: ↑38%**	90 days	49575314
	NS	--	NS	--	NS	--		364 days	49575317
	NS	--	NS	--	NS	--		728 days	49575317

[1]Table compiled by EPA reviewer

Statistically different from control *p<0.05; **p<0.01

NS = not statistically significant

A = absolute liver weight, R = liver weight relative to body weight

Table 20. Liver weight changes in Mice[1]

Parameter	Dose (ppm)							
	30	**50**	**400**	**3000**	**5000**	**10000**	**Duration**	**MRID**
Liver weight	**Males**							
	--	--	NS	--	A: ↑24%* R: ↑14%*	--	7 days	49813533
	NS	--	NS	--	A: ↑37%** R: ↑36%**	--	14 days	49813571
	--	NS	NS	A: ↑20%** R: ↑30%**	--	A: ↑21%** R: ↑62%**	90 days	49575316
	NS	--	NS	--	A: NS R: ↑32%**	--	364 days	49575318
	NS	--	NS	--	A: ↑66% R: ↑95%**	--	655 days	49575318
Liver weight	**Females**							
	--	--	--	--	--	--	7 days	49813533
	--	--	--	--	--	--	14 days	49813571
	--	NS	NS	A: ↑19%* R: ↑25%**	--	A: ↑45%** R: ↑53%**	90 days	49575316
	NS	--	NS	--	A: ↑14% R: ↑42%**	--	364 days	49575318
	NS	--	NS	--	A: ↑24% R: ↑45%**	--	644 days	49575318

[1]Table compiled by EPA reviewer
Statistically different from control *p<0.05; **p<0.01
NS = not statistically significant
A = absolute liver weight, R = liver weight relative to body weight

3. Formation of Adenomas (Key Event 3)

As shown in section II, male rats had statistically significant trends for thyroid follicular cell adenomas at $p < 0.01$. There was also a statistically significant pair-wise comparison of the 1600 ppm dose group with the controls for thyroid follicular cell adenomas at $p < 0.05$. The statistical analyses of the tumors in the male rat study were based upon Peto's Prevalence Test (Table 2). Thus, the tumorigenic dose is 1600 ppm in rats.

Male mice had statistically significant trends, and statistically significant pair-wise comparisons of the 5000-ppm dose group with the controls, for liver adenomas and combined adenomas and carcinomas, all at $p < 0.01$. The statistical analyses of the tumors in the male mouse study were based upon Peto's Prevalence Test (Table 5). Thus, the tumorigenic dose is 5000 ppm in mice.

CARC conclusions for Key Event 3 (formation of adenomas): the CARC agreed that the thyroid tumors observed in male rats and liver tumors in male mice are treatment related.

B. Dose response relationships/temporal associations

The registrant provided the following statements and tables in their mode of action analysis document (MRID 49813573):

"The increased incidences of male mouse liver tumors and male rat thyroid tumors were only observed at the highest dose tested in each of the carcinogenicity studies. Correlated with this tumor occurrence in both cases, the vast majority of the effects associated with CAR activation that were measured in the sub-chronic, chronic and MoA studies were only observed at the same top doses (5000 ppm, mouse; 1600 ppm, rat). Thus, clear threshold dose levels for effects were demonstrated in both mice and rats.".

Also, that "when all three key events were investigated across a range of exposure levels, the weight of evidence indicates that the observed effect (and no-effect or minimal-effect) concentrations of pethoxamid in short and in long-term studies in the CD-1 mouse and Crl CD rat, are entirely consistent with critical events of the most plausible mode of action of CAR activation. In each case, effects in male mice and rats were much more pronounced than effects in females, correlating to the increases in benign tumors that were only seen in males."

Summary of Dose Response (vertically) & Temporality (horizontally) of the Hypothesized pethoxamid Mode of Action in the male mouse.

Dose ppm	Key Event 1 Initiating Event Activation of CAR	Key Event 2 Increased replicative DNA synthesis	Key event: Increased hepatocellular hypertrophy	Key Event 3 Formation of Liver Adenoma
	Time			
Dose				
Timing of observation	Measured at day 7	Measured at day 7	Measured at day 7, weeks. 52 & 95	Measured at week 95
0	- in male CD-1 mouse	- in male CD-1 mouse	- in CD-1 mouse	- in CD-1 mouse
400	- in male CD-1 mouse	- in male CD-1 mouse	- in CD-1 mouse	- in CD-1 mouse
5000	+ in male CD-1 mouse	+ in male CD-1 mouse	+ in CD-1 mouse	+ week 95 in CD-1 mouse

References: Boulet, 2016; McBride, 2016; Harada 2001; Waterson 2000a
- represents no response; + represents a positive response.

Summary of Dose Response (vertically) & Temporality (horizontally) of the Hypothesized pethoxamid Mode of Action in the male rat.

Dose ppm	Key Event 1 Initiating Event Activation of CAR	Key Event 2 Increased replicative DNA synthesis	Key event: Increased hepatocellular hypertrophy	Key Event 3 Formation of Thyroid Adenoma
	Time			
Dose				
Timing of observation	Measured at day 14	Measured at day 14	Measured at day 14, weeks. 90, &.104	Measured at week 104
0	- in male Crl-CD rat	- in male Crl-CD rat	- in male Crl-CD rat	- in male rat
400	- in male Crl-CD rat	- in male Crl-CD rat	- in male Crl-CD rat	- in male rat
1600	+ in male Crl-CD rat	+ in male Crl-CD rat	+ in male Crl-CD rat	+ in male rat

References: Boulet, 2016; McBride, 2016; Waterson 1996
- represents no response; + represents a positive response.

The reviewer created additional tables (Tables 15 and 16) to include all associated and key events identified.

Table 15. Temporal concordance of associative and key events in the proposed MOA- mouse liver tumors						
	CAR activation (Key event 1)	Increased CYP enzymes (associative event)	Increased DNA replicative synthesis/ proliferation (Key event 2)	Increased hepatocellular hypertrophy (associative event)	Increased liver weight (associative event)	Formation of liver adenoma (key event 3)
Doses (ppm)	Timing					
	Not measured	Measured at 7 and 14 days	Measured at 14 days	Measured at 7, 90, 364 and 655 days	Measured at 7, 90, 364 and 655 days	Measured at 655 days
0	ND	No	No	No	No	No
30	ND	ND	No	No	No	No
50	ND	ND	ND	No	No	ND
400	ND	Yes	No	No	No	No
3000	ND	ND	ND	Yes	Yes	ND
5000	ND	Yes	Yes	Yes	Yes	Yes
10000	ND	ND	ND	Yes	Yes	ND

ND = No data

Table 16. Temporal concordance of associative and key events in the proposed MOA- rat thyroid tumors						
	CAR activation (Key event 1)	Increased CYP enzymes (associative event)	Increased DNA replicative synthesis/ proliferation (Key event 2)	Increased thyroid follicular hypertrophy (associative event)	Increased thyroid weight (associative event)	Formation of thyroid adenoma (key event 3)
Doses (ppm)			Timing			
	Not measured	Measured at 14 days	Measured at 14 days	Measured at 7, 90, 364 and 728 days	Measured at 7, 90, 364 and 728 days	Measured at 728 days
0	ND	No	No	No	No	No
25	ND	ND	No	No	No	No
100	ND	ND	ND	No	No	ND
400	ND	Yes	No	No	Yes	No
500	ND	ND	ND	No	No	ND
1600	ND	Yes	Yes[a]	Yes	Yes	Yes
2500	ND	ND	ND	No	Yes	ND
5000	ND	ND	ND	Yes	Yes	ND

ND = No data

[a]The observed increase in BrdU labelling index was not statistically significant

C. Biological Plausibility and Coherence

The registrant submitted the following rationale for biological plausibility and coherence:

"The hypothesized MoA is a well-studied area of investigative toxicology. Chemicals such as phenobarbitone have been shown to cause an increased incidence of hepatocellular adenomas and carcinomas in rats and/or mice through activation of one or more hepatic nuclear hormone receptors e.g., CAR (Lake *et al*., 2014). Activation of CAR is understood to be the first key event in this mode of action. Such chemicals often also result in thyroid adenomas, primarily in the male rat. Further, it has been shown using CAR/PXR knockout mice that the second key event in the mode of action, increased hepatocellular proliferation, cannot proceed in the absence of activation of the receptor (Chamberlain *et al*., 2014). The mode of action studies performed on pethoxamid have provided compelling evidence that an initiating event for the formation of hepatocellular adenomas and carcinomas in rodents is likely associated with the activation of CAR, possibly with weaker interaction with one or more of the other nuclear hormone receptors. For example, activation of the PPARα receptor is associated with the induction of CYP4A isoforms in rats and mice, followed by increased hepatocellular proliferation with the potential for development of liver tumors. Once again, studies with PPAR_ knockout mice have shown that the second and third key events cannot proceed without receptor activation first taking place. The enzyme induction data within the pethoxamid mode of action studies were indicative of primary activation of the CAR receptor by pethoxamid and by phenobarbitone rather than PPARα.

Studies on the development of rodent hepatic neoplasia have shown a direct correlation between the induction of hepatocyte DNA synthesis and the development of altered, hyperplastic, hepatic foci, followed by the development of benign hepatocellular tumors (Holsapple *et al* 2006; Cohen 2010, Elcombe *et al* 2013). For pethoxamid, the second key event within the mode of action framework is increased replicative DNA synthesis which may ultimately lead to the final key event i.e. benign liver or thyroid tumors. Studies using hepatocytes from different species *in vitro* have shown that although increased replicative DNA synthesis occurs in rodents in response to phenobarbitone (and therefore other chemicals eliciting CAR activation), it does not occur, or occurs to a much lesser degree, in humans.

The hypothesized mode of action initiated by the activation of the nuclear hormone receptor CAR is not unique to pethoxamid and has been well studied by many researchers."

D. Alternative Modes of Action

The registrant submitted the following to address potential alternative modes of action:

"Several other modes of action have been associated with rodent hepatic carcinogenesis (Cohen 2010). Such mechanisms can be subdivided into those that involve a direct reaction with DNA and those that do not. Examples of those mechanisms devoid of such DNA-reactivity include those involving AhR, CAR, PXR and PPARα receptor mediated mechanisms, estrogen-mediated, cytotoxicity-mediated and thyroid peroxidase inhibition mediated.

Direct reactivity with DNA: It has been clearly shown in a battery of guideline *in vitro* and *in vivo* genotoxicity studies that pethoxamid is not genotoxic and hence such a mode of action is highly unlikely.

AhR-mediated carcinogenesis: Activation of AhR can lead to similar sequelae of cellular events as that of a CAR mediated effect that can ultimately lead to formation of liver tumors in rodents. For pethoxamid, an AhR-mediated mode of action for formation of adenomas in male CD-1 mice is unlikely because the induction of Cyp1a (a Cyp sub-family that is preferentially induced where AhR activation has taken place) was considerably less than Cyp2b induction as also observed with phenobarbital. Therefore, AhR activation is unlikely to explain pethoxamid-induced liver tumors, and by analogy, an AhR effect is also unlikely to be involved in thyroid tumor development in the male rat.

Estrogen-mediated: Although estrogens have a receptor-mediated mode of action that includes hepatocellular proliferation, it has been proposed that this may be due to the formation of DNA adducts and increased cell proliferation (Cohen 2010). Because there is no structural similarity between pethoxamid and estrogen, a similar MoA of hepato-carcinogenesis is unlikely. Further, there was no evidence of estrogenic activity in the extensive *in-vivo* data package for pethoxamid, notably including the guideline compliant rat two-generation reproductive toxicity study.

Cytotoxicity-mediated: In all sub-chronic and chronic studies, in mouse, rat and dog, there was no evidence of hepatic or thyroid necrosis or general cytotoxicity from either histopathology or clinical biochemistry at any of the dose levels to which animals were exposed. Such pathology would be anticipated to occur if the tumors arose through a cytotoxicity MoA. Instead, a common feature of these studies in rodents was increased hepatocyte hypertrophy, particularly in the centrilobular region. Such an effect is a common feature of liver enzyme inducers, thus supporting the CAR activator MoA being proposed. Therefore, the hypothesized MoA of cytotoxicity is unlikely to play a role in the formation of adenomas in the mouse or rat.

Inhibition of thyroid hormone synthesis: certain chemicals (e.g., anti-thyroid medicine to treat patients with hyperthyroidism) can inhibit the synthesis of thyroid hormones, usually by inhibiting the function of thyroid peroxidase enzymes (enzymes within the thyroid gland responsible for facilitating the coupling of iodine to thyroglobulin – an essential stage in the production of T3 and T4). With reduced thyroid hormone output due to thyroid peroxidase inhibition a negative feedback on the thyroid-pituitary-hypothalamus axis can occur which results in an increase in TSH release, which in turn can lead to thyroid hypertrophy and increased thyroid follicular cell hyperplasia in rats (Redmond O, Tuffery AR 1981). Such a direct inhibiting effect of pethoxamid on thyroid hormone synthesis was investigated in a mechanistic test – "thyroid perchlorate discharge test" (Henderson, 2000). The premise of the study was to characterize what effect pethoxamid may have on free iodine levels (perchlorate which is a competitive inhibitor of iodine uptake by the thyroid was used to discharge free 125iodine from the thyroid gland within the study) within the thyroid gland. Elevated free iodine levels within the thyroid gland are indicative of a failure of iodine organification which can result from inhibition of thyroid peroxidase activity. The model (positive control) thyroid peroxidase inhibitor propylthiouracil was used in the

study. To further differentiate the mechanism at work, another positive control substance was used - phenobarbitone – which acts *indirectly* on the thyroid by inducing hepatic microsomal enzymes including the thyroxine metabolizing UDP glucuronyl transferase. In the thyroid perchlorate discharge test (Henderson, 2000), male SD-CD rats were dosed for 28 days with pethoxamid (1600 and 5000 ppm, diet), phenobarbitone (75 mg/kg/day, oral gavage) or a positive control, propylthiouracil (200 mg/kg/day, oral gavage). Pethoxamid (as phenobarbitone) did not cause a significant discharge of thyroid radioactivity by perchlorate; thus, inhibition of thyroid peroxidase was not occurring with pethoxamid. Furthermore, pethoxamid did not affect the blood T3 level which indicates that it does not inhibit the 5'- mono de-iodonase enzymes (converting T4 to T3). Since there was no decline in TSH levels and no consistent elevation in T3 and T4 levels, it is unlikely that pethoxamid has an agonistic action at the TSH receptor. Comparing the data for pethoxamid with that for propylthiouracil, it suggests that pethoxamid did not directly affect thyroid function. The data obtained for pethoxamid at the high dose level are similar to that for phenobarbitone (TSH levels, thyroid and whole-blood radioactivity); therefore as it relates to liver and subsequently thyroid effects (in the rat), the mode of action of pethoxamid is aligned with that of phenobarbitone. It was concluded (E.U DAR, 2002, 2005) that for the thyroid effects, "the mechanism of action of pethoxamid is similar to that of phenobarbitone.". A direct effect of pethoxamid on thyroid hormone production leading to hyperplasia>thyroid follicular cell adenomas is therefore discounted by the study of Henderson, 2000."

The CARC concluded that alternative MOAs for rats were not fully investigated based on the thyroid hormone data discussed above and the weak BrdU labeling in rat thyroid tissue.

E. Uncertainties, Inconsistencies and Data Gaps

The registrant submitted the following rationale to address uncertainties, inconsistencies, and data gaps.

"Where nuclear receptor activation is predominately restricted to the CAR/PXR receptor, it has been shown in other studies using CAR/PXR knockout mice that the second key event, increased hepatocellular proliferation, cannot proceed. There is good evidence, with respect to this MoA, from dose-response relationships for pethoxamid in male CD-1 mice that in the absence of the initiating event (activation of CAR), the second key event, induction of replicative DNA synthesis, does not occur. Female mice do not show any biologically meaningful increase in tumor incidence compared with males and show correspondingly lesser effects on associative and key events. The same is true in the rat where the increased incidence of thyroid adenomas is restricted to males, which show much larger effects on key and associative events in the liver and thyroid compared with females. It is considered that these data, in conjunction with the lack of evidence for an alternative mode of action for the formation of the liver and thyroid adenomas in the mouse and rat, respectively serve to increase the degree of certainty for this mode of action for pethoxamid induced tumors."

F. Human Relevance

The registrant submitted the following to address human relevance:

"A well-known human drug, phenobarbitone, is typical of rodent hepatocarcinogens that induce tumors by a non-genotoxic mechanism, critically involving hepatocyte hyperplasia. Like pethoxamid, phenobarbital is neither cytotoxic or genotoxic. Induction of some Cyp enzymes, particularly of the Cyp2b family, are diagnostic for phenobarbital and are a consequence of activation of nuclear receptors, particularly CAR but also PXR and to a lesser extent PPARα. Phenobarbital also induces Cyp enzymes in human liver, although there are reports that it may act more through PXR activation than through CAR activation in humans. It is this activation of CAR that is generally viewed as the first key event of phenobarbital-mediated hepatic tumors in rodents, and this event has been reported to occur in both rodents and humans. Human studies, conducted with hepatocytes *in-vitro*, have shown that the second key event, replicative DNA synthesis with hypertrophy/hyperplasia does not occur, unlike rodent hepatocytes.

The evidence indicates that pethoxamid acts as an activator of CAR and based on the difference in biological response in humans and rodents in this context any hepatocellular adenomas developed through activation of these nuclear hormone receptors by pethoxamid in mice are not of relevance to humans. Similarly, the MoA for male rat thyroid adenomas, resulting in the induction of hepatic UGT, leading to increased circulating levels of TSH, is rodent-specific with negligible relevance to humans."

CARC conclusions regarding relevance to humans: Consistent with current HED policy, CAR-mediated tumors are considered relevant to humans.

V. COMMITTEE'S ASSESSMENT of the WEIGHT of the EVIDENCE

The following represents CARC's overall assessment of the data presented at the October 24, 2018 meeting for pethoxamid:

Rats

Based on the rat carcinogenicity study (MRID 49575317):

- With regards to survival, there were no disparities in females and a negative pair-wise comparison of the low dose group with controls in males.
- No significant increase in the incidence of tumors was observed in female rats. Male rats had statistically significant trends for thyroid follicular cell adenomas at $p < 0.01$. Also, there was a statistical difference in pair-wise comparison for adenomas at 1600 ppm ($p<0.05$). Thyroid adenomas were considered treatment-related in males at 1600 ppm, the highest dose tested (HDT).
- Relevant non-neoplastic findings included male thyroid hyperplasia (not significant) at 1600 ppm.
- The CARC concluded that dosing was adequate and not excessive for the carcinogenicity

study in the rat based on the treatment-related tumors as well as thyroid enlargement, thyroid hyperplasia, cystic degeneration and clear cell hepatocytes observed at the highest dose tested in males.

The registrant submitted mechanistic studies and a mode of action (MOA) proposal for pethoxamid-induced rat thyroid tumors through activation of the constitutive androstane receptor (CAR). **The CARC concluded that the submitted data do not adequately support the proposed MOA** based on the following considerations:

- Key event #1 (CAR activation): Increased thyroxine glucuronidation and UGT were observed at 1600 ppm. UGT1A6 mRNA levels were significantly increased starting at 400 ppm. These effects were reversible after a 42-day recovery period.
- Key event #2 (induction of replicative DNA synthesis): BrdU labeling was marginally increased at 1600ppm but this increase was not statistically significant. Thus, the induction of replicative DNA synthesis in considered equivocal. Also, no significant changes in thyroid hormones (T3, T4 or TSH) were reported in the mechanistic studies.
- Key event #3 (formation of adenomas): male thyroid adenomas were observed at 1600 ppm.
- Associative events: In rats, there was a significant increase in thyroid follicular cell hypertrophy at 1600 ppm in males following treatment with pethoxamid for 7 days (MRID 49813533) and 90 days (MRID 49575314). However, no thyroid hypertrophy was observed in the chronic study in rats.

Mice

In a carcinogenicity study (MRID 49575318), groups of 60 CD-1 (Crl:CD-1 BR) mice/sex/dose group were administered pethoxamid (TKC-94; 94.8-95.0% a.i.; Lots TB-960306 and TB 960306C) in the diet at dose levels of 0, 30, 400, or 5000 ppm (equivalent to 0/0, 4.0/5.0, 56.8/68.0, and 982/1068 mg/kg/day in males/females) for up to 95 weeks in males and 92 weeks in females. Interim sacrifice groups of 10 mice/sex/dose group were euthanized at 52 weeks.

- With regards to survival, there were no disparities in females and a negative trend ($p<0.05$) in control males.
- No significant increase in the incidence of tumors was observed in female rats. Male mice had statistically significant trends, and statistically significant pair-wise comparisons of the 5000-ppm dose group with the controls, for liver adenomas and combined adenomas and carcinomas, all at $p < 0.01$.
- Relevant non-neoplastic findings included statistically significant increases in hepatocellular hypertrophy in males and females at 5000 ppm (HDT).
- The CARC concluded that dosing was adequate and not excessive for the carcinogenicity study in the mouse based on the treatment-related tumors in males as well as microscopic findings (swelling/rarefaction of the villous epithelium) in the duodenum and jejunum and decreased body weights in males and females observed at the highest dose tested (limit dose).

The registrant submitted mechanistic studies and a MOA proposal for pethoxamid-induced

mouse liver tumors through activation of the CAR. **The CARC concluded that the submitted data adequately support the proposed MOA** based on the following considerations:

- Key event #1 (CAR activation): Increased PROD was observed at 1200 ($p<0.05$) and 5000 ppm ($p<0.01$), increased CYP mRNA at 400 (CYP2B10; $p<0.05$) and 5000 ppm (CYP1A2, CYP2B10, CYP3A11, CYP4A10; $p<0.05$) and increased CYP activity at 5000 ppm (CYP1A, CYP2B, CYP3A2, CYP4A1; $p<0.05$).
- Key event #2 (induction of replicative DNA synthesis): There was a statistically significant increase in BrdU ($p<0.001$) and PCNA labeling ($p<0.05$) at 1600ppm.
- Key event #3 (formation of liver tumors): male liver adenomas and combined adenomas and carcinomas were observed at 5000 ppm.
- Associative events: liver hypertrophy and increased liver weights were reported in multiple studies.

VI. CLASSIFICATION of CARCINOGENIC POTENTIAL

In accordance with the EPA's Final Guidelines for Carcinogen Risk Assessment (March, 2005), the CARC classified pethoxamid as **"Suggestive Evidence of Carcinogenic Potential"** based on male rat thyroid follicular cell adenomas. There is insufficient evidence to support the proposed thyroid tumor MOA in male rats. However, there is sufficient evidence to support the proposed MOA for mouse liver tumors induced by pethoxamid. There is no concern for mutagenicity in vivo.

VII. QUANTIFICATION of CARCINOGENIC POTENTIAL

Quantification of human cancer risk is not required. The chronic Reference Dose (RfD) will adequately account for all chronic toxicity, including carcinogenicity, which could result from exposure to pethoxamid.

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